A Descriptive study on the Clinical Profile of Snake Envenomation in a Tertiary Care Center in Tamil Nadu and the Diagnostic and Prognostic Utility of Serum Phospholipase A2 in Various Envenomation Syndromes

A dissertation submitted in partial fulfillment of the rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai, to be held in April 2017

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DECLARATION

This is to declare that this dissertation titled — A Descriptive study on the Clinical Profile of Snake Envenomation in a Tertiary Care Center In Tamil Nadu and the Diagnostic and Prognostic Utility of Serum Phospholipase A2 in Various Envenomation Syndromes is my original work done in partial fulfillment of rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai to be held in April 2017.

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Dr. George Abraham

Post graduate Registrar in General Medicine

Department of Medicine

Christian Medical College, Vellore

CERTIFICATE

This is to certify that the dissertation entitled –"A Descriptive study on the Clinical Profile of Snake Envenomation in a Tertiary Care Center In Tamil Nadu and the Diagnostic and Prognostic Utility of Serum Phospholipase A2 in Various Envenomation Syndromes" is a bonafide work done by Dr. George Abraham towards the partial fulfillment of rules and regulations for MD General Medicine degree examination of the Tamil Nadu Dr.M.G.R Medical University, to be conducted in April 2017.

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CERTIFICATE

This is to certify that the dissertation entitled –"A Descriptive study on the Clinical Profile of Snake Envenomation in a Tertiary Care Center In Tamil Nadu and the Diagnostic and Prognostic Utility of Serum Phospholipase A2 in Various Envenomation Syndromes" is a bonafide work done by Dr. George Abraham towards the partial fulfillment of rules and regulations for MD General Medicine degree examination of the Tamil Nadu Dr.M.G.R Medical University, to be conducted in April 2017.

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This dissertation would be incomplete without expressing my gratitude to the people involved in its conceptualisation and completion. First and formost, I would like to thank God Almighty for giving me the opportunity and strength to pursue this topic from start to completion.I express my deep gratitude to all the patients who agreed to participate in this Study.

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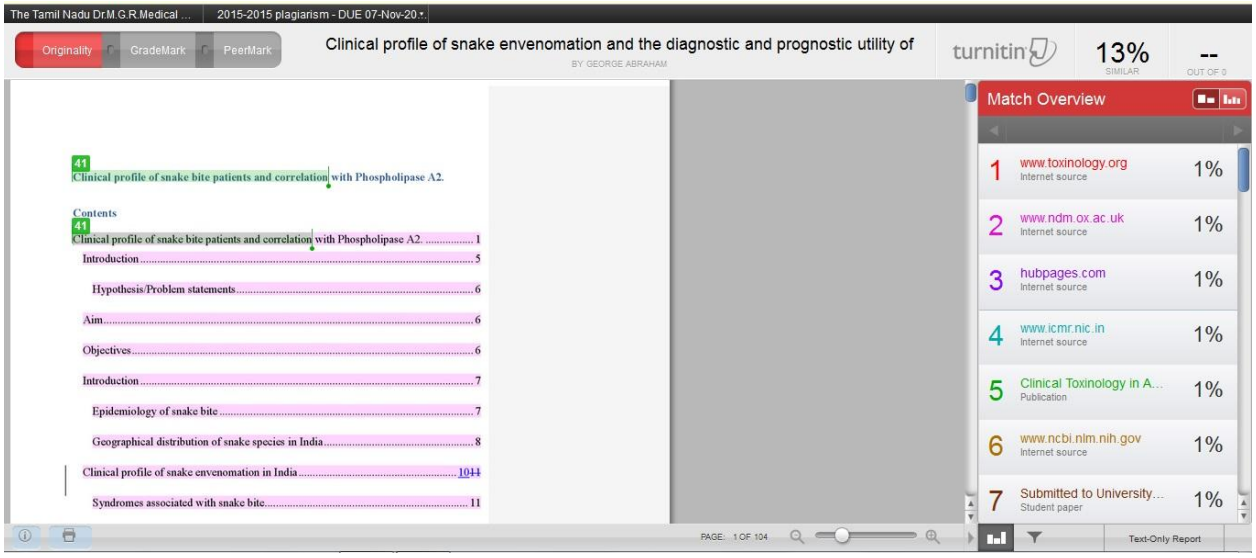

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Dear Dr. George Abraham,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
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Dr. NIHAL THOMAS
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Ref: IRB Min No: 9040 [OBSERVE] dated 04.09.2014

Dear Dr. George Abraham,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Snake venom proteins and envenomation sydromes." on September 4th 2014.

The Committees reviewed the following documents:

1. IRB Application format
2. Curriculum Vitae' of Dr. George Abraham, Dr. Anand Zachariah, Dr. Rajesh Valmiki, Dr. Tunny Sebastian
3. Proforma
4. Informed Consent form (English & Tamil)
5. Information Sheet (English & Tamil)
6. No of documents 1-5

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on September 4th 2014 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Dr. E.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
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We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an 1st Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment following the receipt of the Interim progress/ Annual report and subsequent submission of it to the IRB.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

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May 14, 2016

Dr. George Abraham,
Department of Medicine,
Christian Medical College,
Vellore 632 002

Ref: I. IRB Min No. 9040 dated 04.09.2014

Dear Dr. George Abraham,

The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed the following amendment for the study titled "Snake venom proteins and envenomation syndromes" on April 04th 2016.

(1)Case recruitment (2) Clinical case documentation (3) Laboratory studies (4) Difficulties encountered during isolation of specific venom proteins (4) Materials and Methods (5) Aim (6) Methodology of PLA2 assay (7) Statistical analysis

The following Institutional Review Board (**Blue**, Research & Ethics Committee) members were present at the meeting held on April 04th 2016 at 12.45 am in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC,Vellore	Internal, Clinician

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Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC, Vellore	Internal, Clinician
Dr. RatnaPrabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist

We approve the above amendment as presented.

Yours sincerely,

Dr. Alfred Job Daniel
Principal & Chairperson (Research Committee)
Institutional Review Board.

Chairperson (Research Committee) &
Principal
Christian Medical College
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Clinical profile of snake bite patients and correlation with Phospholipase A2.

Contents

Introduction

Snake bite is one of the neglected tropical diseases. There has been a recent increase the interest in understanding the mechanism of envenomation caused by snake bites. There has been significant geographical variation in the distribution of various snake species in India. Within the individual snake species, the patterns of envenomation differ in various regions of India. This makes it essential to have region based studies on envenomation patterns seen in snake bite patients.

Most of the current guidelines of diagnosis and prognostication of snake bites uses indirect markers which are specific for organ injury caused by various venom proteins in the serum. In order to facilitate earlier diagnosis of systemic envenomation before the development of complications, it is essential to change the focus of diagnosis of systemic envenomation and prognostication to the detection of venom proteins in the serum wherever possible. There needs to be a better understanding of various venom proteins and their possible mechanism of toxicity in vivo. Unfortunately, there have been difficulties in the isolation of different venom proteins. Phospholipase A2 (PLA2) is one the enzymes which has been studied as a marker of envenomation. In this study the role of PLA2 as a diagnostic marker of snake envenomation is evaluated.

Hypothesis/Problem statements

Can serum PLA2 be used as a diagnostic marker for systemic envenomation in snake bite and to assess prognosis of snake bite?

Aim

1. To describe the clinical profile of snake envenomation presenting to a tertiary care hospital in South India.
2. To assess the utility of serum PLA2 in diagnosis of snake envenomation and for assessment of prognosis in snake envenomation.

Objectives

1. To study the clinical profile, temporal course and clinical outcome of snake envenomation presenting to a tertiary care centre in South India.
2. To evaluate serum PLA2 level in diagnosis of snake envenomation.
3. To study the relationship between admission PLA2 levels and the development of complications in patients with snake bite.

Introduction

Snake bite is an important occupational and rural hazard in developing countries in the tropical region.(1)It is one of the most neglected public health issues in Asia. There has been significant underreporting of the snake bite cases and the mortality associated with it. Hence the world wide burden of snake bite is not known.(2) There is significant morbidity, mortality and economic burden associated with snake bite.

Epidemiology of snake bite

The global snake bite initiative has reported that approximately 4,21,000 envenomations and 20,000 deaths occur every year due to snake bites. The actual figures may be as high as 18,41,000 envenomations and 94,000 deaths. (3)

In the rural India, only 7.23% of the snake bite deaths were officially reported. There is wide geographical variation in proportion seeking medical care ranging between 22-75%. In a survey conducted in 30 rural villages in Tamil Nadu, the incidence of snake bite was 3.9% and the mortality was 0.45%. The economic burden on the family as a result of complications of snake bite or delay in treatment was significant and 40% of the victims required to take loan to meet the financial need during the emergency.(4) In the "Million Death Study" all deaths in about 7,000 randomly chosen sample area were subjected to verbal autopsy. Based on the million death study, direct estimate of deaths attributable to snake bite in 2005 was 46,000 (99%CI 41,000-51,000), (1 snake-bite death for every 2 HIV/AIDS deaths). Snake-bites caused 0.5% of all deaths, 3% in 5-14 year-olds. 97% died in rural areas, only 23% in health facilities. The highest numbers of deaths were in

Uttar Pradesh (8,700), Andhra Pradesh (5,200), and Bihar (4,500). In contrast the Government of India's web-site reported an average of only 1,350 deaths/year between 2003 and 2008, and Kastururatne et al., 2008 estimated about 11,000 deaths/year.(5) Hence there was a marked difference in the prevalence of snake bite which estimated by the official registry and direct survey.(5) The highest burden of snake bites occurs in the tropical countries of the South Asia, South East Asia and Sub Saharan Africa.

The large population in rural areas who work outdoors are more prone to snake bites. Men are twice as prone to snake bite as women and the age group commonly affected is between 15-45 years. Agricultural labourers and construction workers are prone to snake bite. Snake bite is a seasonal disease increasing in prevalence during the rainy season between June to September in India.(1)

Geographical distribution of snake species in India

There are about 3000 species of snakes worldwide of which about 450 are found to be dangerous to humans. In India, there are about 216 species of snakes identified of which 52 are found to be venomous. (6)The concept of "Big 4" is used to refer species that cause significant mortality in India: Indian Cobra (*Naja naja*), Common Krait (*Bungarus caeruleus*), Russell's viper (*Daboia russelii*) and the Saw-scaled viper (*Echis carinatus*).(7) Several species outside the big four cause systemic envenomation in specific geographical regions. The most important among these are the hump nosed pit viper envenomation which was first identified and reported from Kerala. It is known to cause severe coagulopathy, acute kidney injury and local reaction. There is no antivenom

available for this snake bite and there was clear inefficacy of Indian Antisnake venom in reversing the coagulopathy caused by Hypnale hypnale. (8) In the Thar desert region of Rajasthan, *Echis sochureki* or severe saw scaled viper was identified recently. It causes severe bleeding manifestations associated with coagulopathy and there is a relative inefficacy of polyvalent ASV to neutralise the same. (9)

The species of venomous snakes that were identified other than the Big 4 in India are described below.

1-**Monocellate cobra (*N. kaouthia*) –** mostly found in North Eastern India, pattern and severity of envenomation similar to that of Naja naja.

2. **Central Asian or Oxus cobra (*N. oxiana*)-** rarely found in the northern parts of India like Kashmir and Himachal Pradesh but no bites reported from India.

3. **Kraits (*Bungarus*)-** Three species identified are Wall's krait (*Bungarus walli*) found in the north east India and the Sind krait (*B. sindanus*) found in Western India. The greater Black krait (*B. niger*) found in Bangladesh is associated with rhabdomyolysis, acute kidney injury and neurotoxicity.

4. **King cobra (*Ophiophagus hannah*)-** This is found in the Western Ghats and north east India. It is the largest and most dangerous snake but there are fewer bite cases and mortality in India.

5.**Pit vipers(Crotalinae)**- The most common species found in India is the the Hump-nosed pit-viper (*Hypnale hypnale*) which is found in the Western Ghats and is associated with local necrosis, bleeding, coagulopathy and acute kidney injury and frequently

misidentified with Echinus carinatus. Malabar pit-viper (*Trimeresurus malabaricus*) of the Western Ghats is associated with local necrosis. The other species of pit vipers found in India include the Bamboo pit-viper (*Trimeresurus gramineus*) of the Western and Eastern Ghats, the Himalayan pit-viper (*Gloydius himalayanus*) found in Western Himalayas, large-scaled pit-viper (*Peltopelor macrolepis*) of the south-west, northern white-lipped green pit-viper (*Cryptelytrops septentrionalis*), red-tailed pit-viper (*C. erythrurus*), mountain pit-viper (*Ovophis monticola*) found in north and north east and arboreal green pit vipers idenitified in North east.(10) Most of these vipers are associated with local swelling with bleeding manifestations and coagulopathy.

6**. Sea-snakes (*Hydrophiinae, Laticaudinae*)-** There have been a few cases reported in the coastal regions of India which is associated with myotoxicity.

The Indian polyvalent anti-snake venom does not neutralise the venom of these snakes(9).

Snake venom is a secreted by salivary glands of the venomous snakes and contains a complex mixture of peptides, glycoproteins and enzymes.(11) These are shown to be associated with the local and systemic envenomation including neurotoxicity, haemotoxicity, myotoxicity, nephrotoxicity and rhabdomyolysis associated with snake bites.(12)

Clinical profile of snake envenomation in India

There has been considerable geographical heterogeneity in the clinical manifestations of snake bite across India.

Syndromes associated with snake bite

1. No envenomation
2. Local swelling only
3. Haemotoxicity with / without local swelling
4. Neurotoxicity only
5. Neurotoxicity with Local swelling
6. Haemotoxicity + Neurotoxicity with / without local swelling
7. Haemotoxicity + Neurotoxicity +renal failure with / without local swelling

Syndrome species correlation in snake bite- Studies so far

The incidence of systemic envenomation occurring in snake bites varies from region to region between 10%-34%.(13) Bites from venomous species can manifest with no systemic envenomation and are referred as dry bites.

Clinical patterns in Viperidae bites

Russell's viper (*Daboia russelii russelii*) presents with local swelling, blistering and occasionally necrosis at the bite site with associated haemostatic manifestations like coagulopathy in 77% of the cases. There were additional features like acute kidney injury and neurotoxicity noted with this species.(14) In contrast, saw scaled viper (*Echis carinatus*) bites are associated with haemotoxicity with local swelling, tissue necrosis and blister formation.(15) The pit vipers bites described in certain parts like Kerala especially the hump nosed pit viper (*Hypnale hypnale*) presents with haemotoxic manifestations like

bleeding, coagulopathy, local swelling and cellulitis with or without acute kidney injury and brown coloured urine.(8)

Clinical patterns in Elapidae bites

In Elapidae, the bites by cobra species especially the Naja naja and N. Kaouthia are associated with neuroparalytic manifestations with extensive local swelling, cellulitis with a high incidence of tissue necrosis and gangrene of the bitten limbs. Elapid bites are characteristically associated with descending paralysis which is progressive in nature.(16) In contrast, the bites of Common krait (Bungarus caeruleus) is classically painless with no features of local envenomation to the extent that the bite site may not be recognised in most cases. Usually victims are bitten while sleeping on the floor. There may be severe abdominal pain mimicking a surgical emergency. The extra-ocular muscles are most commonly involved and ptosis is usually considered as the first sign of early neuromuscular blockade. Subsequently the patients may develop bulbar weakness with difficulty in swallowing, speaking and protrusion of tongue beyond the incisors. As the weakness descends to involve the diaphragmatic muscles, respiratory symptoms develop which can be life-threatening without prompt intubation and ventilation. There is also loss of tendon reflexes and weakness of the limbs noted in a few cases.(17)

According to the WHO algorithm for the South East Asian Countries the following syndromes were defined to correlate with the snake species.

Syndrome 1- Local envenomation with bleeding and clotting disturbances associated with Viperidae

Syndrome 2-Local envenomation with bleeding and clotting disturbabnces with shock or acute kidney injury –Russell's viper

The above symptoms with conjunctival edema and acute pituitary insufficiency has been described with Russell's viper envenomation in Myanmar and South India.

The above symptoms with neurotoxicity like ptosis, external ophthalmoplegia, facial paralysis and dark coloured urine has been described for Russell's viper envenomation in South India, Sri Lanka and Myanmar.

Syndrome 3- local envenomation with paralysis- Cobra /King cobra

Syndrome 4: Paralysis with minimal or no local envenoming:

Bitten on land while sleeping on the ground with/without abdominal pain is associated with krait

Bitten in the sea, estuary or freshwater lakes -sea-snake

Syndrome-5 Paralysis with dark brown urine and acute kidney injury

Above symptoms + bitten on land/bleeding and clotting disturbances- Russell's viper

Above symptoms+ bitten in sea, estuaries without bleeding and clotting disturbances- sea snakes

Clinical features and complications of snake envenomation

The immediate symptoms that arise from snake bite are mostly related to anxiety and fear like chest pain, flushing, palpitations, dyspnoea, syncope and loss of consciousness. Sudden cardiac arrest is also reported very rarely.(18) The occurrence of these symptoms do not relate to envenomation and has a similar incidence irrespective of the species of the snake.

Neurotoxicity associated with snake bite is manifested by ptosis, ophthalmoplegia, bulbar and limb weakness and respiratory muscle including diaphragmatic paralysis. The prevalence of neurotoxicity is much higher in the Northern(19) and Western parts(20) of India when compared to the incidence of haemotoxicity. The most common snakes associated with neurotoxicity are Common krait, Cobra and Russell's viper in certain areas.

Haemotoxicity is associated with bleeding manifestations like bite site bleeding, oral cavity and gum bleeding, internal bleeding manifestations like hematemesis and melaena and hematuria. It is mostly associated with viperine bites which constitute the major envenomation pattern in South India.(21)

The cardiac manifestations occur rarely with viperine bites. The manifestations include tachycardia, bradycardia, atriventricular block, hypotension, pulmonary edema, left ventricular systolic dysfunction associated with myocarditis and cardiogenic shock. Acute coronary syndrome with myocardial infarction associated with snake bite has been reported.(22)

The mechanisms involved in snake bite related nephrotoxicity are multiple including hemorrhage, hypotension, venom induced consumptive coagulopathy, hemolysis, haemoglobinuria and direct venom related nephrotoxicity. The pathological changes observed in the kidney are acute tubular necrosis (found in almost 100% of the biopsy specimens) varying from focal and patchy to diffuse. Acute cortical necrosis is seen in around 25% of the biopsy specimens of snake bite patients with renal failure. Very rarely proliferative glomerulonephritis and interstitial nephritis also has been observed patients

with renal failure. (23)The data on clinic-pathological correlation published previously from our center was based on 19 patients with snake bite induced AKI who underwent renal biopsy. Of the 19 patients, the majority (n=12) had acute tubular necrosis which resolved with a few patients requiring dialysis. 4 patients had normal renal biopsy and the AKI resolved without dialysis in all of them. The patients (n=3) who progressed to develop chronic kidney disease had acute cortical necrosis in the renal biopsy and they subsequently went on to become dialysis-dependent.(24)

Myotoxicity has also been described with Elapidae and viperidae species. It involves the skeletal and cardiac muscles and presents initially as myalgia which progresses to rhabdomyolysis. The myoglobin released into the blood from the damaged muscles gets accumulated in the renal tubules causing acute kidney injury and myoglobinuria. It is characterised by elevation of CPK and LDH. (11) Cardiac muscle involvement typically manifest as myocarditis.(22)

An overview on Daboia russelii / Russell's Viper syndrome

Russell's viper is a common cause of systemic envenmation in India, Sri Lanka and Myanmar.(25)Russell's viper accounts for 30-40% of the patients with systemic envenomation from the Sri Lankan study. More importantly it accounts for the most fatal envenomation by any snake in this geographical region. The clinical syndrome of Russell's viper has been described by WHO for the South Asian countries. The classical presentation includes severe pain and swelling at the bite site with local bleeding. Most patients develop swelling that extends more proximally to involve the joints and can lead

to compartment syndrome and necrotising fasciitis. There is also associated regional lymphadenopathy which could be regarded as a sign of systemic envenomation. Local envenomation is the most common manifestation of Russell's viper envenomation accounting to 91.9% of all the bites. (26)There is associated coagulopathy which manifests as clinical bleeding manifestations like hematuria, haematemesis and melaena which can be often life-threatening. Minor bleeding manifestations include oral and gum bleeding and bleeding from the bite site. Coagulopathy is the second most common manifestation in patients with Russell's viper envenomation accounting for 76.1%.(26) The Indian polyvalent venom was found to be inefficacious in neutralising the coagulopathy in Russell's viper bite in Sri Lanka.(26) There are also case reports of thrombotic microangiopathy developing post Russell's viper envenomation requiring plasma exchange. (27) Renal dysfunction is a characteristic feature of Russell's viper envenomation which can be used to distinguish it from other viper bites like Saw scaled viper. The mechanism of Russell's viper envenomation related nephrotoxocity include shock, venom induced consumptive coagulopathy, intravascular haemolysis, rhabdomyolysis, direct nephrotoxic effect of the venom, ASV related interstitial nephritis and sepsis related secondary AKI. (28) Use of native medications and thereby late presentation to hospital and ASV administration has been one of the predictive factors for Russell's viper related AKI especially in the paediatric population. (28) Nearly one fifth (18.7%) of the patients developed acute kidney injury following Russell's viper bite in Sri Lanka.(26) Abdominal pain is a symptom that is evaluated to have good correlation

with in incidence of coagulopathy and neurotoxicity and its severity correlated with the severity of these syndromes. (26)

Neurotoxicity is a syndrome of Russell's viper that has received considerable attention in view of its intra-species geographical heterogeneity of presentation. It was reported more frequently in patients with Russell's viper bite from Sri Lanka.(29) The most common manifestation of neurotoxicity was ptosis (almost 100%) followed by blurring of vision (93%) and ophthalmoplegia (90%). The incidence of bulbar weakness, respiratory muscle weakness and limb weakness are not reported in the Sri Lankan study. (29) There was no residual neurological deficit noted in any patient at 6 weeks or 6 months. The ophthalmoplegia had the longest duration. Most patients developed neurotoxicity within 8 hours of bite and ptosis resolved within 3 days although ophthalmoplegia lasted for 4-5 days. It was shown that neurotoxicity was associated with larger snakes of Russell's viper species and was associated with higher venom concentrations.(29) The neurotoxicity in Russell's viper is attributed to a pre-synaptic neurotoxin, U1-viperitoxin-Dr1a, which is a phospholipase A2 subtype of venom toxin found in Russell's viper. (30)

Acute pituitary insufficiency following Russell's viper envenomation is a rare entity that has been described in various parts of India, Burma and lately from Sri Lanka. The presentation is hypotension following snake bite without any evidence of sepsis or cardiac failure explaining the shock and responds promptly to intravenous steroids like dexamethasone. (31) The mechanisms proposed for Russell's viper bite related hypopituitarism were pituitary infarct caused by fibrin microemboli as a result of the procoagulant action of the venom toxins and pituitary hemorrhage as a result of

hamorrhagins present in the venom. The hypothalamo-pituitary adrenal axis is involved most commonly in acute pituitary insufficiency following Russell's viper envenomation presenting as hypoglycaemia and hypotension. Steroids are lifesaving in these cases. Chronic pituitary insufficiency involves all pituitary axes and requires hormone replacement.(32) In a prospective study of pituitary failure after Russell's viper bite in Myanmar, of the 9 patients in shock after Russell's viper bites and in 24 individuals who had been severely envenomed, 4 who died had pituitary haemorrhage and 7 who were alive had evidence of hypopituitarism.

Cerebrovascular accident is a rare complication of Russell's viper envenomation. The incidence of large and medium vessel infarct in the brain in Russell's viper envenomation was 1.8% in Srilanka (n=9).(33) The etiology of cerebrovascular accident in Russell's viper envenomation is proposed to be the formation of fibrin microthrombi which may get embolised to the brain. The patients having permanent neurological deficits are associated with higher morbidity and mortality. (33)

Correlation of clinical syndromes to species

In most studies a very low percentage of patients bring the snake that has bitten the patient to the hospital. Therefore the clinical syndrome of the patient is important in trying to identify the potential snake that has bitten the patient. Syndrome species correlation studies are required in different regions to develop algorithms for clinical diagnosis. An example of such a study was conducted by Ariaratnam et al (2009). Such

studies have not been conducted in India and are urgently required in view of the in view of the diversity of snakes and variability of syndromes..

Table 1-Clinical spectrum of syndromes of snake bites, Sri Lanka(34)

Species	**No**	**Local effects(%)**	**Coagulopathy (%)**	**Neuro toxicity (%)**	**Renal toxicity (%)**	**Myo Toxicity (%)**
Russell's viper	319	96	76	59	19	24
Hump-nosed pit viper	302	91	39	_	10	_
Common krait	88	9	_	95	_	_
Cobra	45	91	_	80	_	_

Table-2 Sensitivity and specificity of clinical syndromes as a screening test in identifying snake bites, Sri Lanka

Snake	**Sensitivity (%)**	**Specificity (%)**
Russell's viper	14	100
Cobra	78	96
Common krait	66	100
Hump-nosed viper	10	97

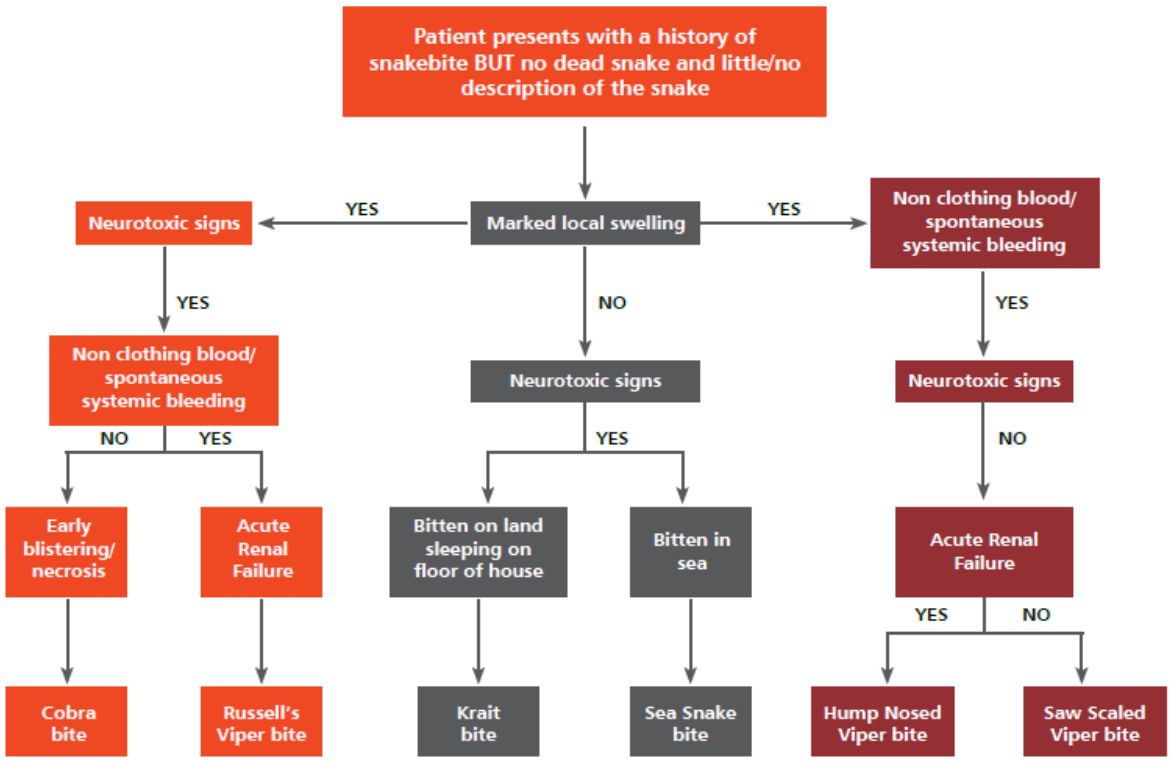


Figure 89a. Algorithm for diagnosis of the snake responsible for a bite in Sri Lanka (Ariaratnam et al., 2009)

Figure 1, from "Guidelines for snake bite management, WHO South East Asia 2016

Figure 1

Management of snake bite patients

First aid and transport

The victim should be reassured and the affected limb has to be immobilised. Searching for the snake, handling of the snake and killing it is not recommended. Accelerated transport to medical care in recovery position is ideal. Tight arterial tourniquet is not recommended. Traditional practices for snake bite management are shown to be more harmful and delay the initiation of ASV.

Anti-snake Venom (ASV)

The polyvalent ASV that is manufactured in India is isolated from the serum of horses after injecting the snake venom by fractionation of the plasma. The polyvalent antisnake venom has antibodies against the Big 4 species of India associated with systemic envenomation namely Russell's viper, saw scaled viper, Common krait and Indian cobra.(35) Although monovalent antivenom is considered more efficacious, the approach across the country has been polyvalent ASV use because the identification of biting species may not be possible.

Dose of anti- snake venom

There is no consensus on the intial dose of ASV that needs to be administered in patients with systemic envenomation. The usual practice is to administer 10 vials with careful monitoring of signs of hypersensitivity reaction and anaphylaxis.(36) The patient should be closely monitored and reassessed every 4-6 hours for resolution of envenomation symptoms. If there is persisting neurotoxicity or haemotoxicity, then the dose is repeated.

There have been several trials comparing low dose ASV(6 vials initially) to high dose ASV(12 vials initially) in patients with systemic envenomation which showed no statistical difference in the resolution of envenomation syndromes or outcome of patients between the two groups.(37) However these studies were of small sample size, did not confirm biting species and had limitations in design. A larger well designed RCT was

conducted in Nepal where they compared slow administration of ASV (2 vials initial push followed by 4 vials every 4 hours for 12 hours and 2 vials for the next 2 hours) against 10 vials in the first hour of presentation. This trial showed no significant difference in the outcome in both the arms in the resolution of envenomation symptoms and the incidence of antivenom reactions (in Publication references in WHO guidelines for South East Asia 2016).

A systematic review published in 2015 on the various trials on high and low dose ASV concluded that there was no significant difference in the resolution of haemotoxicity, neurotoxicity, incidence of acute kidney injury, duration of hospital stay or mortality between low and high dose ASV regimens. However there was a significant cost effectiveness associated with the low dose regimen.(38) There is urgent need for well conducted dose finding studies in India to establish the exact dose for Antisnake venom.

Problems and limitations of anti-snake venoms

The success of antivenom treatment depends of the ability of the antibodies to bind, neutralise and eliminate the toxins injected into the body and thereby reversal of the clinical and laboratory manifestations of snake envenomation. The polyvalent ASV is able to neutralise the circulating antibodies but there is controversial evidence on the reversal of tissue and end organ damage.(19) There has been a clinical inefficacy of polyvalent ASV in reversing the neurotoxicity of Krait envenomation requiring higher doses in patients with Krait bite.(19) This is probably because of irreversible binding of

neurotoxic venom proteins to presynaptic nerve terminal causing nerve terminal degeneration.

In a study of Russell's viper bite in Sri Lanka 30% of definite and 40% of probable Russells' viper envenomation required more than 20 vials of antivenom. In a randomised controlled trial comparing Haffkine equine polyspecific antivenom to a new monospecific ovine Fabantivenom (Polonga TAb) in Russell's viper envenomation, it was found that 70% of Russell's viper envenomation was neutralised with 10 vial of Haffkine's polyvalent antivenom but it was associated with high rates of hypersensitivity.

In South India our clinical experience has been that there is clinical inefficacy of polyvalent antivenom in Russell's viper envenomation. However there are no published studies which corroborate this.

The geographical variation in the snake venom toxins and antigenic properties across India also leads to variable efficacy of ASV. The source of venom for ASV manufacture is primarily obtained from the Irula cooperative in Mammallapuram. (39)

There are serious adverse events associated with the use of polyvalent ASV like pyrogenic reactions, hypersensitivity reactions, anaphylaxis and late onset serum sickness. This limits the administration of ASV in patients with severe envenomation. Low dose adrenaline administered subcutaneously is found to be effective in the treatment of anaphylaxis due to ASV.(40) The other issues of ASV include the irregular,

insufficient supply and its high cost Indian setting where most of the victims are from the low socioeconomic strata. (41)

Local envenomation

According to the WHO guidelines, ASV should be administered for swelling and pain extending more than half the bitten limb within 48 hours or rapid progression of swelling beyond wrist/ankle within few hours, swelling after bite on digits and regional lymphadenopathy. Immobilisation with limb elevation is advisable. Antibiotics are indicated if there is local cellulitis/necrotising fasciitis. The latter may also require surgical debridement.

Haemotoxicity

The indication for ASV administration include spontaneous systemic bleeding manifestations, coagulopathy defined as positive non clotting whole blood clotting time more than 20 minutes or INR >1.2 or prothrombin time 4-5 seconds longer than the control time or thrombocytopenia of <1,00,000 /mm3. The parameters are repeated every 6 hours and if there is persistence of coagulopathy or systemic bleeding, then ASV further dose of ASV is given. Persistence of coagulopathy beyond 20 vials of ASV may warrant the use of blood products like fresh frozen plasma or cryoprecipitate.

Neurotoxicity

Any neurotoxic manifestation at admission, bilateral ptosis, external ophthalmoplegia and paralysis requires ASV at admission and the patient has to be reassessed every 6 hours for resolution of signs and symptoms. Evidence of respiratory muscle weakness like diaphragmatic and intercostals muscle weakness will require mechanical ventilation.

Acute kidney injury

The patient may have oliguria, anuria, hemoglobinuria, myoglobinuria, rising creatinine/urea levels, evidence of rhabdomyolysis and intravascular haemolysis. ASV has to be administered for these situations and the patient needs to be monitored for the need for renal replacement therapy.

Venom proteins responsible for clinical manifestations

The toxic component of snake venom is classified into enzymes, polypeptides, glycoproteins and compounds with low molecular weight. There are at least 26 different enzymes that are detected in snake venom of which 12 enzymes are found to be common in all species of snakes.(42) The enzymes include fibrinogenolytic enzymes such as Alpha-fibrinogenases, Beta-fibrinogenases, and Gamma-fibrinogenases. Other enzymes include plasminogen activator releasers such as Ecarin, prothrombin activator, prothrombinase complex formation inhibitors such as Phospholipase A2,B,C and D, Factor X-activator, Factor V-activator, Factor XI-activator, Protein-C-activator, fibrinogenolysin, Platelet aggregation inducers, either without coagulant activity, or with

coagulant activity ,Platelet aggregation inhibitors, such as alpha fibrinogenases or 5-Nucleotidase, or ADPase, or fibrinogen receptor antagonists, Von Willebrand factor-dependent platelet aggregation inducers. Zinc metalloproteases, which disrupt the endothelial lining of blood vessels causing spontaneous bleeding, hyaluronidases (spreading factor), arginine esterases and, L-amino acid oxidases which is widely found in snake venoms, and is responsible for the yellow coloration of snake venom due to the presence of riboflavin as a prosthetic group(43)

L-amino acid oxidase acts by causing oxidative deamination of the amino acids, inhibition of platelet aggregation, induction of apoptosis, hemorrhagic effects and cytotoxicity. The other enzymes in snake venom include phosphodiesterases, phosphatases, choline- esterases, transaminases, proteases, 5- nucleotidases, esterases, ATPase, RNAase which account for toxicity which are not part of acute envenomation.(44)

This is a summary of the few major primary compounds that are detected in the venom of some of the most common families of snakes in South Asia and their probable mechanisms of actions.

Table 3 showing the various venom proteins and their proposed mechanisms of action

Type of compound	**Action on body**	**Snake family**
Acetyl choline esterases AchE	Tetanic paralysis	Colubridae, Elapidae
Arginine esterases	Believed to predigest prey	Viperidae
Bradykinin potentiating peptides	Pain, hypotension, immobilize prey	Viperidae
C- type lectins	Modulate platelet activity, prevent clotting	Viperidae
Cysteine rich secretory proteins	Believed to induce hypothermia and immobilize prey	Colubridae, Elapidae,Viperidae
Disintegrins	Inhibit platelet activity and promote hemorrhaging	Viperidae

Hyaluronidases	Increase interstitial fluidity aiding in the dissemination of venom proteins	Elapidae, Viperidae
L-Amino acid oxidases	Cell damage, apoptosis	Elapidae, Viperidae
Metalloproteinases	Hemorrhage, myonecrosis, believed to predigest prey	Atractaspididae, Colubridae, Elapidae, Viperidae
Myotoxins	Myonecrosis, analgesia , immobilise prey	Viperidae
Nerve growth factors	Believed to cause cell apoptosis	Elapidae, Viperidae
Phosphodiesterases	Causes hypotension and shock	Colubridae, Elapidae, Viperidae
Phospholipases A2(PLA2)	Causes myotoxicity, myonecrosis, damage to call membranes	Colubridae, Elapidae, Viperidae
PLA2 based presynaptic neurotoxins	Immobilises prey	Elapidae, Viperidae
Prothrombin activators	Disseminated intravascular coagulation (DIC)	Elapidae
Purines and pyrimidines	Hypotension, paralysis, apoptosis, necrosis, immobilisation of prey	Elapidae, Viperidae
Sarafotoxins	Myocardial ischemia, increased blood pressure, disturb heart rhythm	Atractaspididae

Serine proteases	Disrupts hemostasis, hypotension, immobilize prey	Colubridae, Viperidae
Three finger toxins (3FTx)	Rapid immobilisation of prey, paralysis and death	Colubridae, Elapidae

Venom proteins responsible for specific envenomation syndromes

Each envenomation syndrome was attributed to multiple venom proteins.

Table 4 demonstrates the correlation between specific enveomation syndrome and venom proteins.

Specific envenomation syndromes	**Venom proteins responsible**
Swelling and bruising	Venom endopeptidases, metalloproteinase haemorrhagins, phospholipases, endogenous autacoids like histamine, serotonin and kinins.
Hypotension and shock	Oligopeptides and vasodilating autacoids
Platelet and coagulation abnormalities	Venom phospholipases, Zinc metalloproteinases.
Neurotoxicity	Phospholipase A2, alpha and beta bungarotoxins, acetylcholine esterases.
Myotoxicity	Phospholipase A2 myotoxins, and metalloproteinases
Acute kidney injury	Venom phospholipase A2 and metalloproteases

Increased capillary permeability	Metalloproteases.

Diagnostic tests for snake envenomation

1. 20 minute whole blood clotting time test (20WBCT)

It is a useful, informative and bedside test with almost no requirement for trained personnel. Absence of clotting of a 2ml blood sample in a snake bite patient at 20 minutes indicates the presence of coagulopathy. In South East Asian countries, this is the most simple test that can distinguish Viperidae bites which are associated with coagulopathy from dry bites and Elapidae bites.

False positive results in 20WBCT is seen in cases where the tube is made of materials other then glass like plastic, polypropylene, polystyrene and in glass containers which are washed with detergent which prevents activation of Hageman factor on contact of glass with the blood. Hence the glass used for clotting tests has to be washed with 0.9% normal saline only.

There are false negatives associated with 20WBCT. A non clotting 20WBCT is predictive of a fibrinogen concentration of <0.5g/l with more than 90% sensitivity and specificity.(45) However, mild coagulopathy may be missed and this may result in

delay in ASV administration. Hence WHO recommends ASV administration in the presence of clinical bleeding manifestations even with normal 20WBCT and periodic monitoring of 20WBCT to detect delayed envenomation. Coagulation parameters like PT, APTT and fibrinogen needs to be measured if venom induced consumptive coagulopathy is suspected.

2 .**Enzymatic assays for diagnosis of snake envenomation- A review of Phosholipase A2**

Phospholipase enzymes

Phospholipases are enzymes that break down or hydrolyse the glycerophospholipids. Depending of the site of hydrolysis, the are divided into 5 types namely, phospoholipase A1 which hydrolyses at the sn-1 site and phospholipase A2 at the sn-2 site. Phospholipase B acts at both these sites. Phospholipase C acts on the glycerophosphate bond where as phospholipase D removes the polar head group. (46)

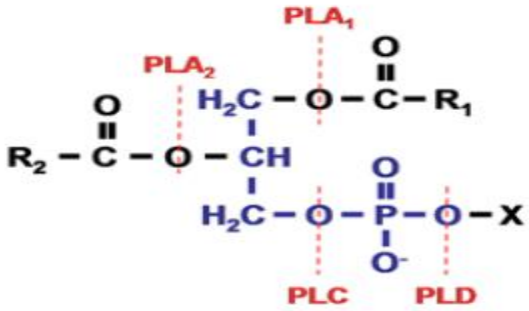


Figure 2 depicting the site of cleavage of different phospholipase enzymes.

Phospholipase A2 as predictor of systemic envenomation

It has been described that the serum phospholipase levels are elevated in viperidae and elapidae bites when compared to patients with no envenomation.(47) The need for a rapid and reliable diagnostic test to detect snake envenomation particularly in the context of limited availability of antisnake venom and the high rates of allergy and anaphylaxis.(48) The clinical relevance is that most of the current decisions and protocols on ASV administration are strictly based on clinical features and development of complications after which ASV may not have much role. It is hence necessary to detect systemic envenomation at an earlier stage to enhance appropriate early administration of ASV in snake bite patients.

Mechanism of action of PLA2

Phospholipases are a enzymes which are capable of calcium 2+ ion dependent hydrolysis of the phospholipids of the cell membrane of the cells cleaving phosphoglycerides into free fatty acids and lysophospholipids. This enzyme is found in all forms of life and has a very significant role in regulation of phospholipid turn over at the cell membrane, the maintenance of membrane fluidity and trafficking, cell maturation and maintenance. It is also involved the regulation of normal apoptosis and the eicosanoid production involving prostaglandins and leukotrienes. Hence this enzyme is also implicated in the structural damage of the cell and the spread of venom resulting in local and systemic

envenomation. The hydrophilic region of the cytotoxin gets surrounded by the released fatty acids which permit the cytotoxin to be released from the enzyme-cytotoxin complex facilitating the spread of cytotoxin.(49) There is destruction of phospholipids on the extracellular as well as cytoplasmic side promoting cell death. There is concomitant endogeneous lipase stimulation and it is not known whether the cytotoxic effect could be fully attributed to exogeneous PLA2 of the snake venom. There is myotoxicity associated with PLA2 which is inflammatory in nature mediated by the enzymatic stimulation of arachnoid acid metabolites and non-catalytic nociceptive effects.(50) The inflammatory action of phospholipases has been shown to be associated with increase in vascular permeability.(51) The active catalytic form MT III(Myotoxin III) with Asp at 49^{th} position and the inactive catalytic form of PLA2, MT II(Myotoxin II) with Lysine variant at 49^{th} position are shown to have phagocytic and cytotoxic effects.(52)

The venom PLA2 has affinity to the synaptic cleft and acts presynaptically to cause destruction of the motor nerve terminal due to its enzymatic activity. This is one of the proposed mechanism that accounts for the neurotoxicity in patients with snake bite. The factors that mediate the binding of PLA2 to the synaptic cleft are not fully understood. (53) The hemotoxicity due to PLA2 is due to its antiplatelet aggregating activity, anticoagulant and hemolytis action. Increase in vascular permeability is associated with hypotension. Other pharmacological actions include bactericidal effect and stimulation of inflammatory response.(49)

PLA2 is a superfamily of enzymes with almost 15 subgroups. The 4 main types include

PLA2 (s PLA2), cytosolic PLA2(c PLA2), calcium independent PLA2 (i PLA2) and lipoprotein associated PLA2 (Lp PLA2) of which the secreted PLA2 is found mostly in the snake venoms.(54)

Biochemical properties of phospholipase A2

The venom derived s PLA2 is further divided to 4 subtypes, types 1,2,3 and 4. Types 1 and 2 are derived from snakes and 3 and 4 are of special biological interest derived from other species like Gila monster but not from snakes. (46) These secreted PLA2 was found in the venom of sea snakes, crotalids, vipers and elapids. There are individual variations in the composition of different secretory PLA2 in the venom of these different snakes. The venom of old world snakes are almost similar to the secretory PLA2 of group 1a, but has an additional C terminal tail and different organization of disulphide bonds hence referred to as 2b sPLA2. A subgroup of this protein called 2b sPLA2 with a lysine instead of aspartate at 49 th position has decreased catalytic activity and calcium binding but has high levels of cytotoxicity and myotoxicity. This is found exclusively in viperids.(46)

The beta bungarotoxin which is a major toxin responsible for the neurotoxicity in kraits has 2 chains of which chain A is formed by a type 1 s PLA2. Hence phospholipase A2 is found in different combinations in the venoms of different snakes.

PLA2 in different snake species in India

There are about 20 different toxic enzymes known which are attributed to systemic envenomation in snake bites and PLA2 is one among them. PLA2 is one of the constituent of the snake venom found in Elapids and *Viperidae*.(55) Since it is only one of the enzymes present in the venom, it may not attribute to all the clinical manifestations of patients with snake bite. However, in the Indian scenario where most of the venomous bites are contributed by *Viperidae* and *Elapidae* family of snakes, assessment of PLA2 levels may be of a diagnostic significance.

Geographical variation in the composition and potency of venom proteins with specific reference to PLA2

There is considerable variation in the composition of different venom proteins in different parts of India. Venoms of Russell's viper in north and western India showed presence of 3 protein bands with molecular weight of 9000, 39,000 and 66,000 in the SDS PAGE(Sodium dodecyl sulphate/polyacryl gel electrophoresis) which were absent in the South Indian species. The phospholipases were acidic in the north and western snakes and was alkaline in the South Indian species. Higher phospholipase activity had correlation with higher lethal potency.(56)

The venom of *naja naja* species has different biochemical and pharmacological properties in different regions of India. It was observed that there was a high mortality associated with the cobra bites in Eastern India despite giving adequate antivenom. It was

postulated that the variations in the toxicity and lethality of cobra bites were due to the variations in the peptides and phospholipase A2 in different areas. There was proven organ specific toxicity differences with more vasculotoxicity and myotoxicity in mice models of the venom of *Naja naja* obtained from Eastern India when compared to Southern and Western India.(57) The variation in envenomation and potency was further explained by mass spectrometry analysis which showed absence of 12kDa, 24 kDa and 33 kDa peptides in the venoms obtained from South India and the presence of a 6.7 kDa peptide which was found exclusively in the venom obtained from Eastern India.(58) There is also considerable variation in the phospholipase A2 fractions which are not fully characterized as well as the three finger toxins in the venom of cobra species.(59)

PLA2 in the serum and its diagnostic utility in systemic envenomation

Most of the diagnostic tests in snake envenomation currently used are indirect methods of organ sytem injury caused by snake bites like coagulopathy, neurotoxicity and renal failure. The disadvantage of these tests is that they are generally detected after the establishment of systemic envenomation. There is a need to establish a diagnostic test which is based on the detection of venom proteins of the snake which could predict systemic envenomation. PLA2 is present in viperidae and crotalidae families which makes it really significant in Indian setting where the species of these families constitute most of the envenomation.(47)

A good diagnostic test for snake bite should have the following characteristics: (a) good sensitivity and specificity, (b) cost effectiveness, (c) point of care bedside test.

Maduwage et al, showed that there was a statistically significant difference in the PLA2 activity in the subjects with snake bite and systemic envenomation when compared to their non envenomated counterparts. The maximum PLA2 activity was seen for Russell's viper envenomation (median-55.7 μmol/ml/min), mild increase was noted for hump nosed pit viper (median-13.6 μmol/ml/min), cobra (median-14.8 μmol/ml/min), krait (median-17.2 μmol/ml/min) compared to (median-6.0 μmol/ml/min) in non envenomated samples.(47)

Elevated PLA2 and associated medical conditions

PLA2 is normally essential for the integrity of cell membranes and is present in the serum in normal individuals. However, it is also an acute phase reactant protein and becomes elevated in certain medical conditions. PLA2 activity is increased in sepsis, pancreatitis, pancreatic acinar cell tumors, peritonitis, multiple injuries, rheumatoid arthritis and other arthropathies, malignancies, complications of pregnancies and post-operative states.(60) Enzymatic tests for PLA2 do not distinguish between the different PLA2 enzymes and between human and snake venom PLA2. Local inflammation and organ system dysfunction in snake bite could also contribute to increased endogenous PLA2 levels. Hence it is not clear if PLA2 can be used a diagnostic test for snake bite.. PLA2 can cause renal injury but the mechanism is unclear. It is proposed that there is accumulation

of lysophospholipids and long chain acyl carnitines and long chain acyl coenzyme A which is produced by the increased catalytic activity of phospholipase A2 in ischemic renal injury which inhibit Na+K+ ATPase of the proximal tubule resulting in renal injury.(61) PLA2 can be elevated in kidney disease. Primary membranous nephropathy is another condition where higher levels of antibodies to phospholipase A2 receptors were associated with higher disease activity. (62) PLA2 can be cause and consequence of renal injury. The significance of elevation of PLA2 in snake bite related renal injury is unclear.

Immunological diagnostic tests

The principle behind immunological tests is that the venom antigen in the serum is coupled with a known antibody and the extent of antigen antibody complex formation is detected by using an enzyme linked to the known antibody which produces a colour change. The use of ELISA (enzyme linked immunosorbent assay) has been attempted in the detection of snake venom antigens in the serum. However there has been cross reactivity, non specific reactivity and variation in the quality of reagents that were used which hindered the bedside applicability of these tests.(63) Subsequently, ELISA tests were developed against the venom antigens of the Big 4 snakes of India which was used to identify the venom present in the tissues of autopsy patients who died of snake bite.(64)

In Australia, a snake venom detection kit was designed to detect the presence and type of specific venom protein of the poisonous species of snakes. The best method was sample collection from the bite site and the kit could identify nanogram quantities of venom. If

bite site was not identified, then blood or urine was used in patients with systemic envenomation. If dead snake is available, a swab from the fangs may yield a positive result.(65) This test is used in the field to diagnose snake bite in the field and to guide monovalent antivenom use. No other country presently uses venom detection tests for diagnosis of snake bite.

3 DNA diagnosis from the bite swab

Attempts have been made to diagnose biting species PCR-aided DNA sequencing from swabs of bite sites. This is a useful research tool but has low yield and is costly.(66)

Characteristics of a good diagnostic test

Currently there is no diagnostic test for systemic envenomation based on venom protein detection. A good diagnostic test in snake bite has to be

Rapid

Reliable diagnosis of envenomation

Bed side point of care testing

Enable Biting species diagnosis

Cost effective

Should not be altered by ASV administration (47)

Problems in the development of diagnostic test for snake envenomation in Indian setting

Currently the diagnosis of snake envenomation in India is based on clinical assessment and monitoring of systemic envenomation for haemotoxicity and neurotoxicity, 20WBCT/PT/APTT for the diagnosis of venom induced consumptive coagulopathy and monitoring of urine output and renal parameters for the incidence of complications. These factors largely determine the decisions on ASV administration in patients with systemic envenomation. There is no laboratory diagnostic test identified that predict systemic envenomation before the incidence of complications in snake envenomation that could facilitate early administration of ASV.

There is geographical variation in the prevalence of snake species across India. There is also variation in envenomation syndromes of each snake species across geographical regions due to variability in the composition of venom proteins. This makes it difficult to formulate a diagnostic test based on venom proteins that is effective in diagnosis of snake envenomation all over the country. Hence there is a need for diagnostic tests formulated based on the regional prevalence of different snake species and envenomation patterns.

The enzymatic assays do not distinguish endogenous and exogenous proteins. Cross reactivity between human proteins and venom proteins poses difficulty for immune diagnosis. The concentration of venom proteins in the serum is very low and hence the tests need to be very sensitive.

Various attempts at Christian Medical College have been made to detect venom proteins based on immunostaining for secondary antibodies in the serum on ASV coupled columns and SDS-PAGE (Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis). The venom proteins could not be successfully detected in these attempts due to the low concentration of venom proteins in the serum and the cross reactivity of the venom proteins with human serum proteins and ASV.(unpublished data).

The review of literature highlights the need for developing a snake bite diagnostic test as well as the problems in developing such a test. The present study is to evaluate the role of PLA2 enzyme level in diagnosis of snake envenomation.

Lacunar knowledge and justification

(1) Clinical study of snake envenomation syndromes in Tamil Nadu

There has been considerable geographical variation in the envenomation syndromes across different regions of India. This is partly attributed to the geographical variation in snake species as well as the regional variations of venom proteins within a snake species. The information on the local envenomations syndromes in Tamil Nadu is limited where there is predominance of Viperidae bites and specifically Russell's viper envenomation. Russell's viper envenomation has been noted to have high rates of complications including haemotoxicity in combination with acute kidney injury and neurotoxicity

leading to mortality. Hence it is essential to understand the patterns of envenomation in South India and the response to antivenom.

(2) Serum PLA2 as a diagnostic test for snake envenomation

The diagnostic utility of PLA2 has been documented in a previous study by Maduwage et al. The utility of this test needs evaluation in the Indian setting.

Materials and Methods

The study protocol was defined at the beginning of the study and was approved by the Institutional Review Board (IRB). In view of the difficulties in isolating the venom proteins, an addendum was added to assess the diagnostic and prognostic utility of a specific protein Phosholipase A2 which was also approved by the IRB.

This is a descriptive single center study done in patients above the age of 15 who present to Emergency Department of our hospital with history of snake bite/ unknown bite with typical envenomation syndromes. Informed consent was taken from the patient/relatives at the start of recruitment.

Patient population:

Snake envenomation

Inclusion criteria

Patients who presented to Emergency Department of CMC Vellore with alleged history of snake bite with envenomation syndromes at presentation or at a later stage and also in patients with suspected envenomation syndromes without the history of bite or bite marks who had been noted to have treatment response with antisnake venom from September 2014 to January 2016.

All patients above the age of 16 years with informed consent by the patient or the relatives (if the patient is unconscious/sedated)(informed assent from caretakers if 16-18 years of age) will be recruited in the study.

Exclusion criteria

Patients who had not given informed consent, paediatric age group(<15 years), patients who are brought dead to triage with alleged history of snake bite or envenomation syndromes, patients admitted with snake bite with no signs of envenomation syndromes after observation for 24 hours will be excluded from the study.

Snake bite without envenomation- snake bite without evidence of local or systemic envenomation.Since the number of non-envenomated snake patients coming to our center was less, the non envenomated control samples (n=64) were obtained after informed consent of the patient/relatives from Government Vellore Medical College.

Normal healthy controls for PLA2 measurement- 30 staff and student at CMC Vellore

Exclusion criteria: All patients/relatives who did not provide consent and pregnant women were excluded from the study.

Detailed clinical assessment was done by the principal investigator to assess the envenomation syndromes at admission and on a daily basis till the discharge or for 5 days whichever is earlier.

Blood samples: The venous samples were collected every day from admission for a maximum of 5 days depending upon the duration of stay of the patient. The samples were immediately refrigerated at <4 degree Celsius. The serum is isolated after centrifugation and analysed for the levels of PLA2.

The outcome was assessed by the mortality, incidence of various envenomation syndromes and its duration of persistence after the administration of ASV incidence of complications like renal injury, venom induced consumptive coagulopathy, requirement of product support like Fresh frozen plasma and cryoprecipitate to neutralize the coagulopathy.

Definition of envenomation syndromes

Local envenomation

Local swelling in the absence of a tourniquet

Enlarged tender lymph node draining the bitten limb

Necrosis, blistering

Necrotising fascitis

Compartment syndrome –absent pulses

Gangrene

Haemotoxicity

Hemorrhagic manifestations (systemic bleeding menifestations) or

Whole blood clotting test › 20 minutes with a clean dry new test tube, never reuse the test tube.

Deranged PT/PTT (INR >1.2 or PT prolonged by more than 4-5 seconds)

Platelet count <100,000 cells/mm3

Neurotoxicity- (any of the below)

Ptosis or Opthalmoplegia, neck muscle weakness, bulbar weakness – dysphagia, difficulty in speaking, inability to protrude the tongue beyond the incisors

Limb muscle weakness grade 4 or less

Respiratory paralysis-Reduced single breath count<10, paradoxical breathing / Type 2 respiratory failure/need for mechanical ventilation, respiratory arrest at presentation

Definition of Renal failure- RIFLE/AKIN criteria

The RIFLE criteria is used for the diagnosis of AKI in snake bites. This is as follows

Risk – 1.5-fold increase in the serum creatinine, **or** glomerular filtration rate (GFR) decrease by 25 percent, **or** urine output <0.5 mL/kg per hour for six hours

Injury – Twofold increase in the serum creatinine, **or** GFR decrease by 50 percent, **or** urine output <0.5 mL/kg per hour for 12 hours

Failure – Threefold increase in the serum creatinine, **or** GFR decrease by 75 percent, **or** urine output of <0.3 mL/kg per hour for 24 hours, **or** anuria for 12 hours

Loss – Complete loss of kidney function (eg, need for renal replacement therapy) for more than four weeks

ESRD – Complete loss of kidney function (eg, need for renal replacement therapy) for more than three months

The AKIN diagnostic criteria for AKI specify an abrupt (within 48 hours), absolute increase in the serum creatinine concentration of ≥0.3 mg/dL (26.4 micromol/L) from baseline; a percentage increase in the serum creatinine concentration of ≥50 percent; or oliguria of <0.5 mL/kg per hour for more than six hours.

Impending AKI signalled by abrupt reduction in kidney function over 48 hours: decreasing/no urine output, increased/increasing serum creatinine concentration, clinical "uraemia syndrome" (nausea, vomiting, acidotic breathing, hiccups, fetor, drowsiness, confusion, coma, flapping tremor, muscle twitching, convulsions, pericardial friction rub, signs of fluid overload). Patients with any of these features should be monitored for other clinical signs of "uraemic syndrome", pulse rate, postural blood pressure, height of

jugular venous pulse, respiratory rate, temperature, auscultation of lung bases for crepitations, fluid balance chart and/ or daily weight.

Most patients with AKI become oliguric (urine output < 400 ml/day or < 30 ml (children <0.5ml/kg bodyweight /hour).

Cardiac toxicity

Cardiac involvement has been described for viperdae bites and involves visual disturbances, dizziness, faintness, collapse, shock, hypotension, cardiac arrhythmias, myocardial damage.

Cardiac involvement is studied with serial Blood Pressure monitoring, ECG and Echocardiography if there are clinical signs of congestive cardiac failure.

Generalised increase in capillary permeability is manifested as facial, periorbital, conjunctival oedema (chemosis), bilateral parotid enlargement, pleural and pericardial effusions, pulmonary oedema, massive albuminuria and haemoconcentration.

Antivenom reactions

Antivenom reactions are classified into 3 categories.

1. Early anaphylactic reactions occur characteristically within 180 minutes of the start of the antivenom. It can have diverse manifestations like itching, urticaria to bronchospasm, shock and angioedema. It is mediated by complement activation by IgG or Fc segment of the immunoglobulins of the antivenom.

2. Pyrogenic reactions occur within 1-2 hours after the start of ASV characterized by fever with chills, rigors, fall in BP and shock due to vasodilation. The pyrogen contamination during the manufacture is attributed as the probable cause.
3. Late serum sickness to ASV occurs after a week of ASV administration characterized by fever, nausea, vomiting, diarrhoea, itching, recurrent urticaria, arthralgia, myalgia, lymphadenopathy, periarticular swellings, mononeuritis multiplex, proteinuria, immunecomplex nephritis and occasionally encephalopathy.

Treatment variables that were assessed

Local measures-tourniquet, pressure immobilisation

Antibiotics

Anti-snake venom dose

Total dose administered to patient including dose administered in outside hospital

Adrenaline/hydrocortisone

Neostigmine

Ventilation /bagging

Dialysis requirement

Transfusion requirement and blood product support

ICU care

Treatment outcomes

Haemotoxicity

Time to normalisation of CT or PT/PTT

Neurotoxicity

Time to regaining normal power (except respiratory muscle weakness)

Time to extubation

Allergic reactions

Minor-itching, urticaria, wheals

Major-angioedema, anaphylactic shock, death

Mortality

Need for surgical debridement or amputation

Outcome of the patient- discharge/death/discharged against medical advice

Mortality analysis based on (1) pathophysiology leading to death; (2) Death as a result of complication of snake bite; (3) Anaphylaxis; (4) Deaths unrelated to snake bite.

Methodology of PLA2 assay

Sample Preparation

Envenomated and non-envenomated serum samples were diluted in the ratio of 1:100 using NOBA buffer contains 100 mM Tris, 10 mM Cacl2, 0.1 M Nacl pH 8.0.

Assay Procedure

PLA2 activity was assayed on the chromogenic substrate 4-Nitro-3-ocatanoyloxy benzoic acid (NOBA).

Twenty five µl (25 µl) of each diluted serum samples were mixed with 200 µl NOBA buffer contains 100 mM Tris, 10 mM Cacl2, 0.1 M Nacl pH 8.0 and 25 µl of NOBA substrate with final substrate concentration of 0.3 mM.

Then the plates were incubated at 37 ˚c for 60 min, and absorbance was recorded at 405 nm in ELIZA reader.

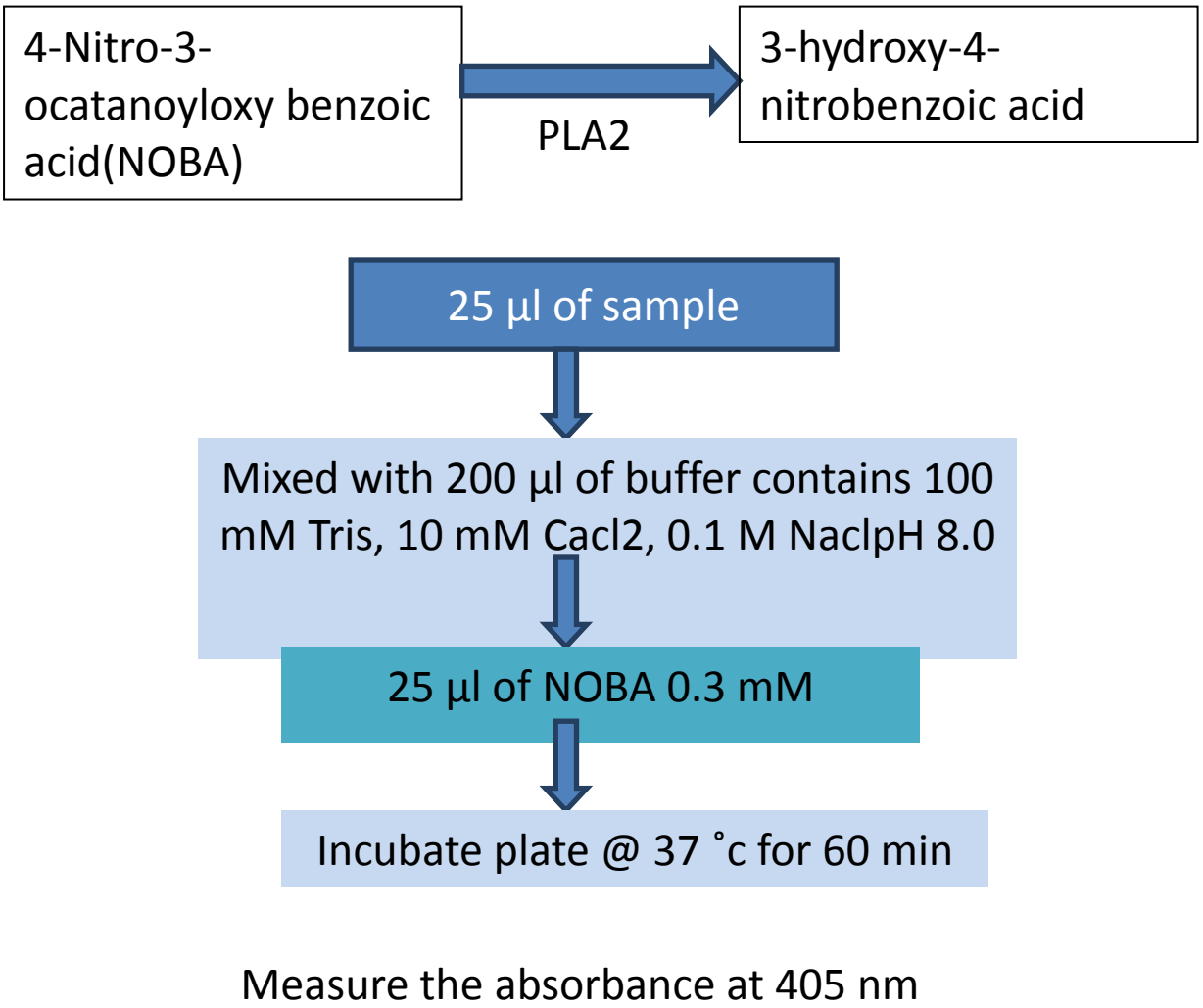
Validation of the optical density values

All the samples were assayed in triplicates and the mean value obtained in optcal density was taken for analysis. If there was high variation in the 3 values, the assay was repeated again in triplicates and the mean value was taken for analysis.

Flowchart

Figure 3-

flowchart



The optical density values obtained is converted to s PLA2 activity using the following formula

sPLA2 Activity (µmol/min/ml) = OD x 0.200 ml x Sample dilution /10.66 X 0.01 ml

10.66 is the extinction co-efficient of the bi product (DTNB)

0.200 ml is the volume of the reaction mixture

sample dilution = 100

0.01 ml is the sample volume

Analysis of the clinic-laboratory correlation of snake bite with PLA2 levels

The PLA2 levels in serum were measured at admission in Optical density using spectrophotometry. It was done for 100 patients with systemic envenomation and 64 non envenomated controls (Patients with snake bite without systemic envenomation). The PLA2 values in μmol/min/ml was obtained by multiplying the optical density values with a constant 187.62 based on the dilution used in the lab and the volume of serum.

Sample size calculation

As per the Australian Snake Bite study Project 11, which was a study aimed to correlate the venom proteins in the serum with the clinical profile of snake bite patients, considering the incidence of coagulopathy as 61% among the patients with systemic envenomation syndromes in red bellied black snake bite, with precision of 10% and confidence interval 95%, the sample size calculated was 92 .

Type of data and method of analysis

The principal investigator fills up a questionnaire containing history, demographic details, clinical examination findings with the treatment details at admission and daily for a period of 5 days or till discharge. The phospholipase activity is measured on the serial serum samples taken as mentioned in the methodology.

The data was analyzed using simple descriptive methods such as frequencies, box plot, histograms etc to screen for outliers and data validation (ranges etc). The prevalence of overall mortality rate was presented with 95% CI. Similarly the incidence of syndromes was presented with 95% CI as overall. . The correlation/association between syndromes, socio demographic variables and mortality, complications was done using chi-square test and also was presented with Relative Risk with 95% CI. The correlation between various envenomation syndromes and the phospholipase A2 in serum at admission was analysed using tests of significance like p value comparing it to the PLA2 levels in non envenomated controls. The relation of incidence of complications and the admission PLA2 levels was assessed for significance using p values among envenomated patients.

Funding and approval

The protocol and the amendment were approved by the institutional review board and the funding was provided by the FLUID grant of the IRB.

Results

The clinical assessment and documentation was carried out for a period of 2 years from February 2014 to January 2016. 167 patients fulfilled the inclusion criteria and were enrolled for the study after obtaining informed consent.

STROBE FIGURE

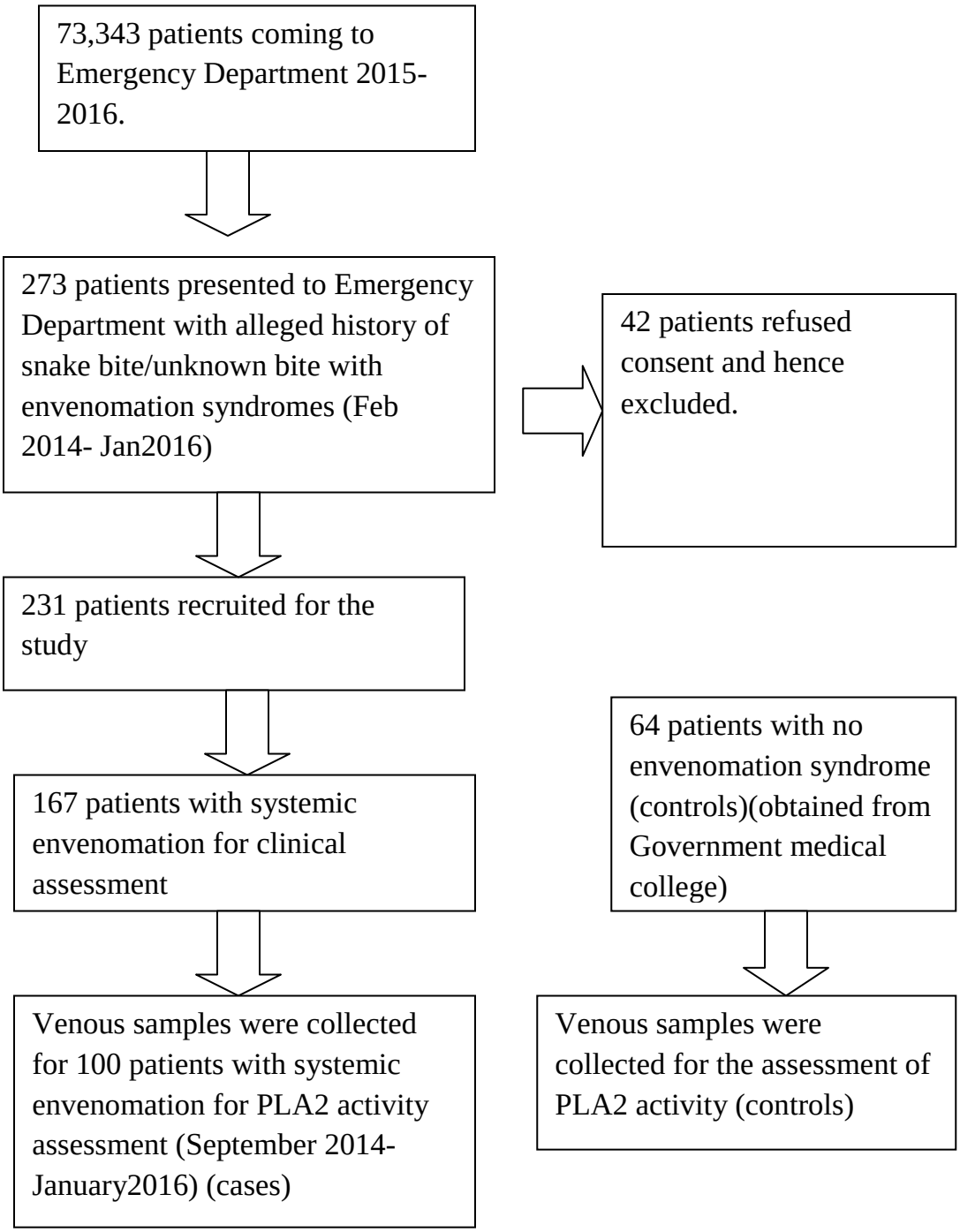


Figure 4-STROBE figure

STRUCTURE OF RESULTS SECTION

SECTION I- CLINICAL PROFILE OF SNAKE ENVENOMATION

Demography of patients (Figure 5)

The median age of the patients was 36 years (15-68). Two thirds of the affected were males. Agricultural labourers constituted majority of the population (64%) followed by construction labourers including masons (21%) followed by housewives.

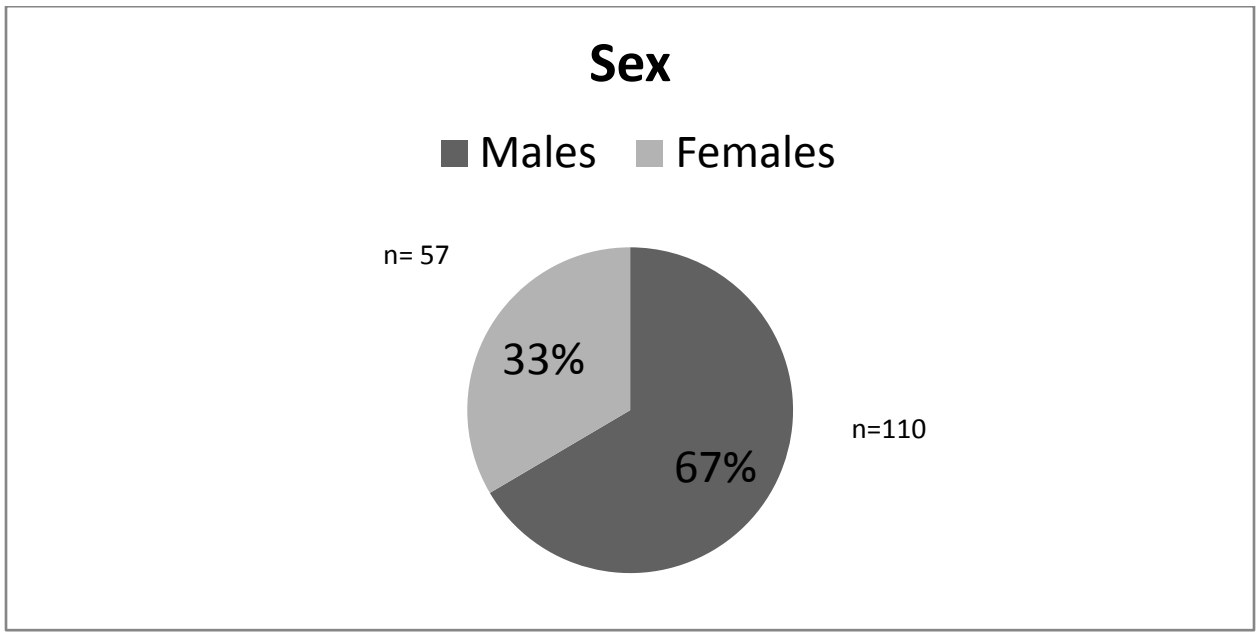


Figure 5 Snake bite- sex distribution

Site of bite (Figure 6)

Most of the bites were in the lower limbs. Right side upper and lower limbs had slightly higher bites compared to left side. There were 2 patients who had typical envenomation syndromes of snake bite but the bite site could not be identified. These patients had pure neurotoxicity.

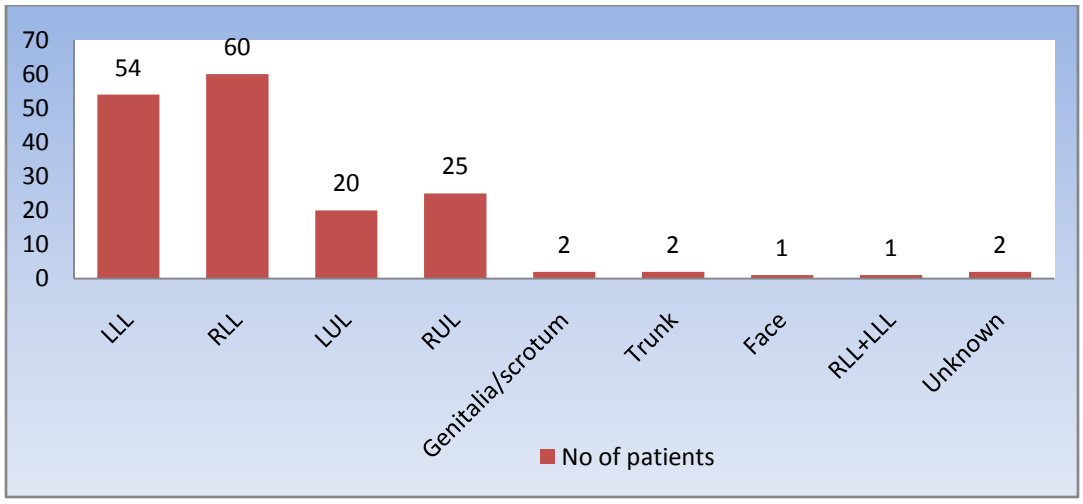


Figure 6- Distribution of site of snake bite

The time delay from snake bite to ASV

The median time gap between snake bite and ASV administration was 4 hours (range 1-185 hrs). There were 12 cases where the time lapse between first ASV dose and admission was not available.

Abdominal pain in Russell's viper envenomation

The incidence of abdominal pain in Russell's viper envenomation was 15.83% n=19. In patients with Russell's viper envenomation with abdominal pain at presentation, 84.2% n=16 developed VICC, 78.9% N=15 developed neurotoxicity and 63.2% N=12 developed AKI.

Distribution of various envenomation syndromes in snake bite patients (Table 5)

The categorisation of syndromes was based on the WHO South East Asia Guidelines (2016) (See Review of literature). Russell's viper bite syndromes include haemotoxicity or haemotoxicity in combination with neurotoxicity and/or acute kidney injury. Pure haemotoxicity could either be due to Saw Scaled viper bites or Russell's viper. Krait bite syndrome causes neuroparalysis without local swelling and may have associated abdominal pain. Cobra bite syndrome causes neuroparalysis with local swelling.

There were 3 patients (1.79%) with no envenomation and 6 patients (3.58%) with local envenomation. There were 26 patients (15.57%) with local swelling and haemotoxicity (probably Saw scaled viper or Russell's viper envenomation). There were 102 patients with combinations of haemotoxicity with neurotoxicity and/or acute kidney injury (61%) (which were probably due to Russell's viper envenomation). The most common Russell's viper syndromes were haemotoxicity with neurotoxicity and haemotoxicity with neurotoxicity and acute kidney injury. There 12 cases (7.2%) with neurotoxicity without local swelling (probable Krait bite) and 18 cases (10.8%) with neurotoxicity with local swelling (probable cobra bite). All the deaths occurred in the syndromes of Russell's viper with haemotoxicity with AKI.

Therefore in this study Russell's viper is the most common species of snake responsible for systemic envenomation. The distribution of the patterns of envenomation is shown in Table 5.

Table 5- Snake envenomation syndromes

Envenomation syndromes	Pattern of toxicity	Number of patients	Percentage of patients	Number of deaths
No envenomation		3	1.79	
Local swelling only		6	3.58	
Haemotoxicity+local swelling	Saw scaled viper or Russell's viper envenomation	26	15.57	
Haemotoxicity without local swelling		0	0	
Haemotoxicity+Neurotoxicity+local swelling	Russell's viper envenomation (61.07%)	41	24.55	
Haemotoxicity+Neurotoxicity without local swelling		3	1.79	
Haemotoxicity+Neurotoxicity+renal failure+local swelling		43	26.06	3
Haemotoxicity+renal failure+local swelling		15	8.98	2
Neurotoxicity only	Common Krait bite	12	7.19	
Neurotoxicity +local swelling	Indian cobra bite	18	10.78	

Correlation of Envenomation Syndrome and identification of dead snake species (Table 6)

Among the patients who brought the snake to the hospital, further identification of the snake species was carried out in Forensic Medicine Department with assistance from a trained herpetologist and was correlated with their clinical syndrome. 13 snakes were brought of which two were non-venomous. The syndrome species correlation were consistent with known literature:

(a) Haemotoxicity with associated neurotoxicity and/or acute kidney injury syndrome – Russell's viper envenomation

(b) Neurotoxicity in the absence of local swelling -Krait envenomation

(c) Neurotoxicity and local swelling - Cobra bites

(d) Haemotoxicity and local swelling- Saw scaled vipers presented

There is absence of neurotoxicity and acute kidney injury in Saw scaled viper bite.

Table 6 Correlation of Envenomation Syndrome and identification of dead snake species

Snake species identified	Number	Syndromes (n)
Saw scaled viper*(Echis carinatus)*	2	Local envenomation (1) Haemotoxicity+local envenomation (1)
Russell's viper (*Daboia russelii*)	4	Haemotoxicity+neurotoxicity+local envenomation (2) Haemotoxicity+local cellulitis(1) Haemotoxicity+ AKI+ local enveomation(1)
Indian Cobra (*Naja naja*)	3	Local envenomation (1) Neurotoxicity + local envenomation (2)
Common Krait (*Bungarus caeruleus)*	2	Neurotoxicity without local envenomation (2)
Russell's kukri	1	Local envenomation (1)
Indian Wolf snake	1	No envenomation (1)

Neurotoxicity in snake bite patients (Figure 7)

The proportion of neurotoxicity in snake bite patients was 67.07% (n=112). The common neurological manifestations seen were ptosis and ophthalmoplegia. Severe neurological manifestations like respiratory muscle weakness including diaphragmatic paralysis, bulbar weakness and limb weakness were relatively less common (about 35%).

The median duration of ptosis and ophthalmoplegia were 3 days (range 1-8 days) and 4 days (range 2-10 days) respectively. The median duration of bulbar and respiratory muscle weakness was 2 days (range1-5 days). The occurrence of various manifestations of neurotoxicity in snake bite is outlined in Figure 8.

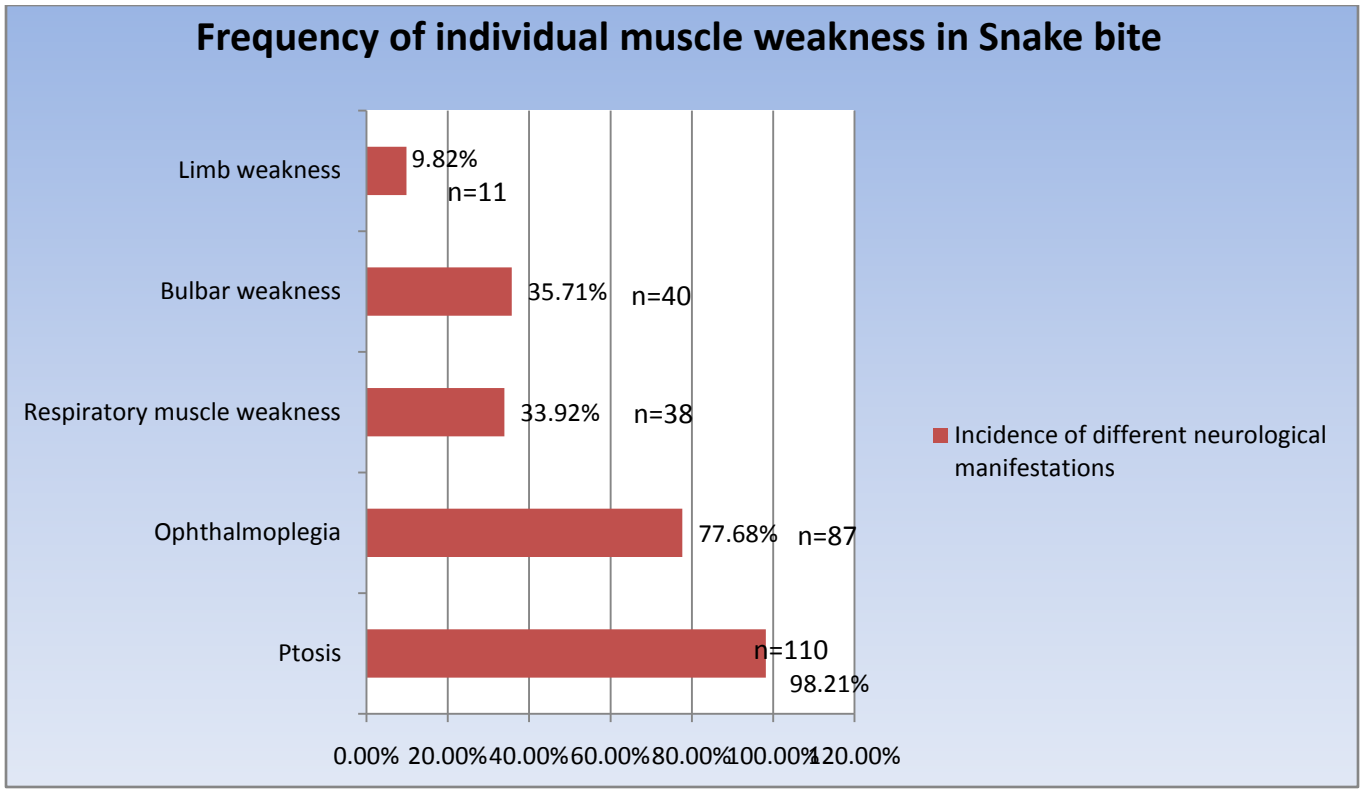


Figure 7- **Frequency of individual muscle weakness in Snake bite**

<u>Differences in neuroparalysis spectrum</u> (Table 7)

Ptosis and opthalmoplegia occurred in the majority of both Russell's viper envenomation and Elapidae syndromes. Russell's viper envenomation syndrome was associated with lower frequency of bulbar, respiratory and limb weakness.

The occurrence of ptosis and ophthalmoplegia did not differ between viperine envenomation and neurotoxic envenomation in the absence of haemotoxicity (krait and cobra bites) $p>0.999$. However the occurrence of respiratory muscle weakness and limb weakness were higher in krait and cobra envenomation compared to viperine envenomation with $p=0.051$ and $p= 0.007$ respectively. There was a trend to increased

occurrence of bulbar weakness in the krait/cobra group compared to viperine group although not statistically significant p=0.357. The occurrence of respiratory muscle weakness (p=0.019)and limb weakness (p=0.0135)was significantly higher in krait envenomation than viperine envenomation as shown in table 7.

The duration of neurotoxicity was measured as the time for complete resolution of any weakness. The neurotoxicity without local reaction as observed in the classical Krait Bite lasted for a median of 4 days (Range 3-7 days). The classical Russell's viper envenomation which was very common in the area had neurotoxic manifestations lasting for a median of 4 days (Range 1-6 days). The neurotoxicity with cellulitis without haemotoxicity were rapidly reversible with ASV (probable cobra envenomation) with a median duration of 3 days (1-5 days).

Table 7 Neuroparalysis spectrum in snake bite syndromes.

Pattern of neurotoxicity	Probable Russell's viper syndrome (Haemotoxicity + Neurotoxicity)		Probable Krait bite (Pure neuroparalysis)		Probable Cobra Bite (Neuroparalysis +local swelling)	
	n (%)	Median duration	n (%)	Median duration	n (%)	Median duration
Ptosis	81 (98.78%)	3 days	12 (100%)	3 days	17 (94.4%)	2 days
Ophthalmoplegia	63 (76.82%)	4 days	10 (83.3%)	4 days	13 (72.2%)	3 days
Bulbar muscle weakness	26 (31.71%)	2 days	6 (50%)	3 days	7 (38.9%)	2 days
Respiratory muscle weakness	23 (28.05%)	Not assessed	8 (66.67%)	Not assessed	7 (38.9%)	Not assessed
Limb weakness	5 (6.1%)	2 days	4 (33.33%)	3 days	4 (22.2%)	1 day

Haemotoxicity in snake bites (Figure 8)

Haemotoxicity was the most common manifestation of systemic envenomation due to snake bite occurring in 76.05% of the patients (n=127). Most of the cases presented at our centers had internal bleeding manifestations like hematuria, haematemesis and melaena (63.78%, n=81). The most common manifestation of haemotoxicity was venom induced consumptive coagulopathy which occurred in 76.38% of the patients with haemotoxicity (n= 97). 66.1% of patients had thrombocytopenia. More than half of these patients had reversal of coagulation parameters with the administration of ASV. 35.43% (n=45) of the patients with haemotoxicity required product support for the normalization of coagulation parameters. The occurrence of various haemotoxic manifestations are as shown in figure 8.

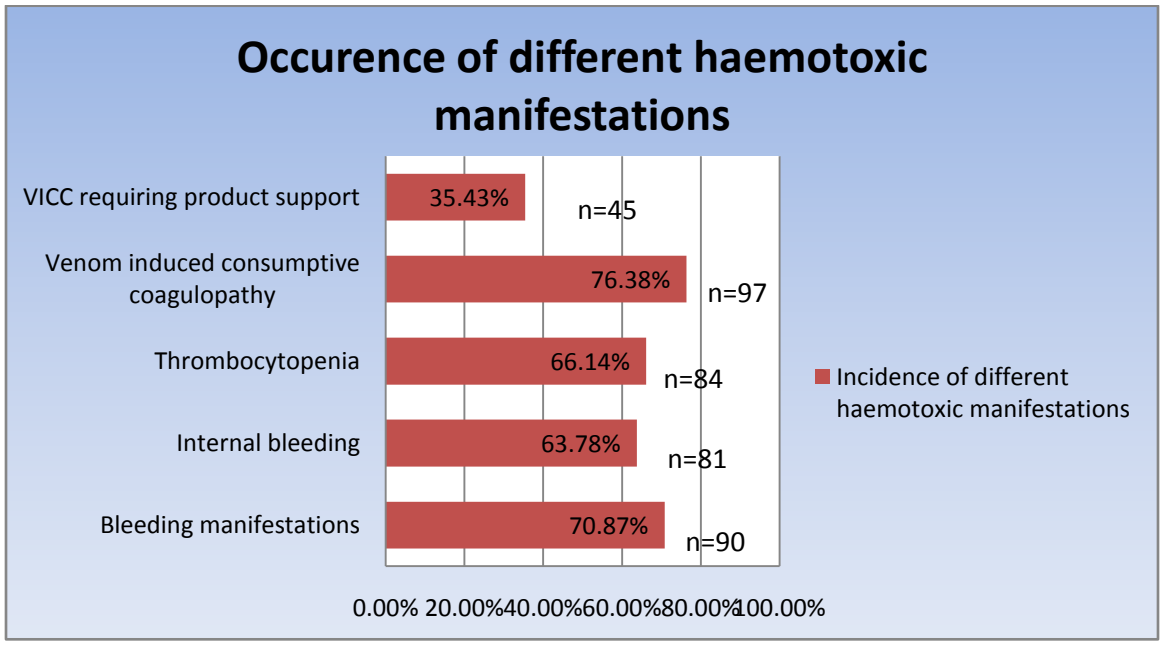


Figure 8 Occurence of haemotoxic manifestations (denominator is the patients with haemotoxicity).

The median duration of haemotoxicity was 24 hours (6 hours to 5 days). It was seen that most of the patients had reversal of coagulation parameters and bleeding manifestations after the administration of ASV with in 1 day. Venom induced consumptive coagulopathy which required product support involving fresh frozen plasma and cryoprecipitate required longer duration for reversal of haemotoxicity.

Acute Kidney Injury

The incidence of acute kidney injury was 34.73% (n=58). Just more than half of the patients with acute kidney injury n=34 (20.36%) required haemodialysis and the rest showed resolution of renal functions without the requirement of renal replacement therapy. 14.97% (n=25) of the patients showed a triad of hemolytic anemia, thrombocytopenia and acute renal injury but the diagnosis of microangiopathic hemolytic anemia and hemolytic uremic syndrome was not established in most of them. Few patients underwent renal biopsies (n=3) the histopathology of which showed acute tubular necrosis. Rhabdomyolysis as defined by muscle injury with elevation in the CPK levels more than 5 times the upper limit of normal (>975 IU/ml)(67) was seen in 26.41% of the patients(n=34) Most of the patients were lost to follow up after the discharge but among the patients (n=10) who came for review, there was resolution of renal function to normal levels with a median duration of 90 days.

Muscle injury following snake bites

Muscle injury was diagnosed if the patient developed severe muscle pain or elevated CPK more than 5 times the normal (CPK>975 IU/ml). 47 patients out of 132 (35.6%)

developed myotoxicity among total patients with snake bite. In Viperidae bites, 44.85% developed muscle injury (n=48). In patients with Russell's viper syndrome, muscle injury was documented in 45.05% (n=41). In patients with pure haemotoxicity, muscle injury was documented in 43.75% (n=7). Among the patients who developed AKI, 48.39% (n=30) had features of rhabdomyolysis. The incidence of muscle injury in Krait and cobra bites were 8.33% (n=1) and 5.55% n=1 respectively.

Local envenomation

88.02% (n=147) had local envenomation associated with swelling at the bite site. Most of them (82.03%, n=137) had swelling tenderness and redness with probable cellulitis requiring antibiotics. The incidence of necrotizing fasciitis requiring debridement and fasciotomy was 7.78% (n=13).

Anti-snake venom (ASV) (Figure 9)

About three fourth of the patients (75.45%, n=126) who presented to CMC had received ASV from outside. Most of them had received liquid ASV with a median dose of 8 vials (Range 1-30). The median total ASV vials given per patient was 16 (range 2-45). One third of the patients (33.54%, n=54) who received ASV had some form of hypersensitivity reaction. The frequency of hypersensitivity reactions were: itching (94.54%), urticaria (74.55%) , anaphylaxis (29.09%) and bronchospasm (36.36%) (see figure 9).

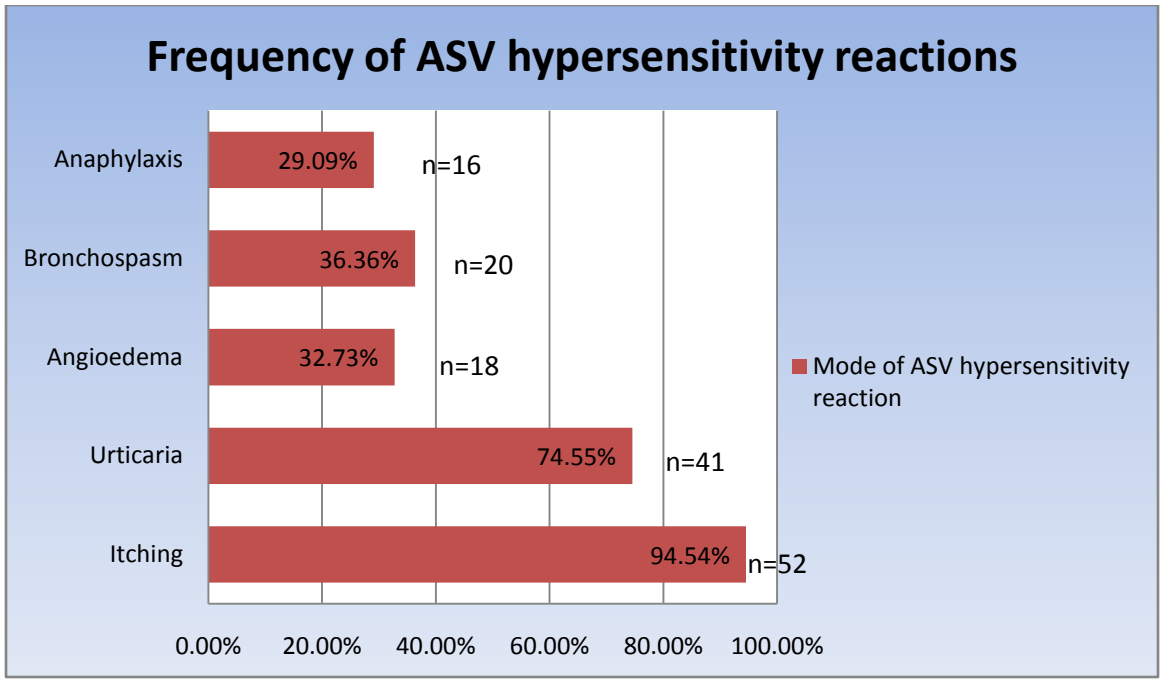


Figure 9 Frequency of hypersensitivity reaction to ASV

(The denominator is the number of patients who developed ASV hypersensitivity n=54)

ASV dose requirement in different envenomation syndromes

Russell's viper envenomation syndrome required maximum ASV for reversal of toxicity with median of 18 vials in comparison to pure hemotoxicity of 14 vials, Krait bite syndrome of 13.5 vials and Cobra bite syndrome of 11 vials.

Table 8 summarises the ASV dose requirement in different envenomation syndromes.

Envenomation syndrome	**Median ASV dose in vials**	**Range**
Russell's viper	18	(0-44)
Pure haemotoxicity (Saw scaled/Russells viper)	14	(9-30)
Viperine envenomation	17	(0-44)
Krait	13.5	(10-22)
Cobra	11	(5-32)

The median ASV requirement in Elapidae bites at our center was 12.5 vials (5-32) and that of Viperidae bites were 17 vials. This difference was statistically significant (p=0.002).

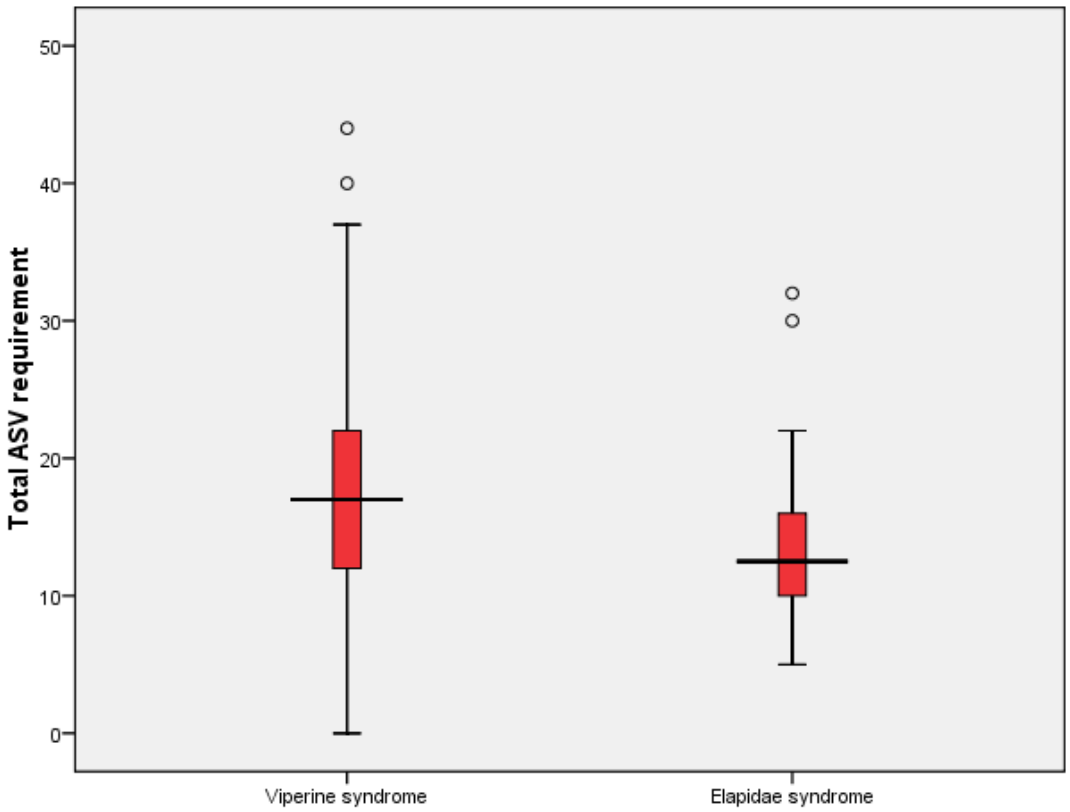


Figure 10 showing the increased requirement of ASV for Viperine syndrome compared to Elapidae bites

The patients with Russells viper envenomation required higher dose of ASV when compared to patients with pure haemotoxicity. (p=0.012)

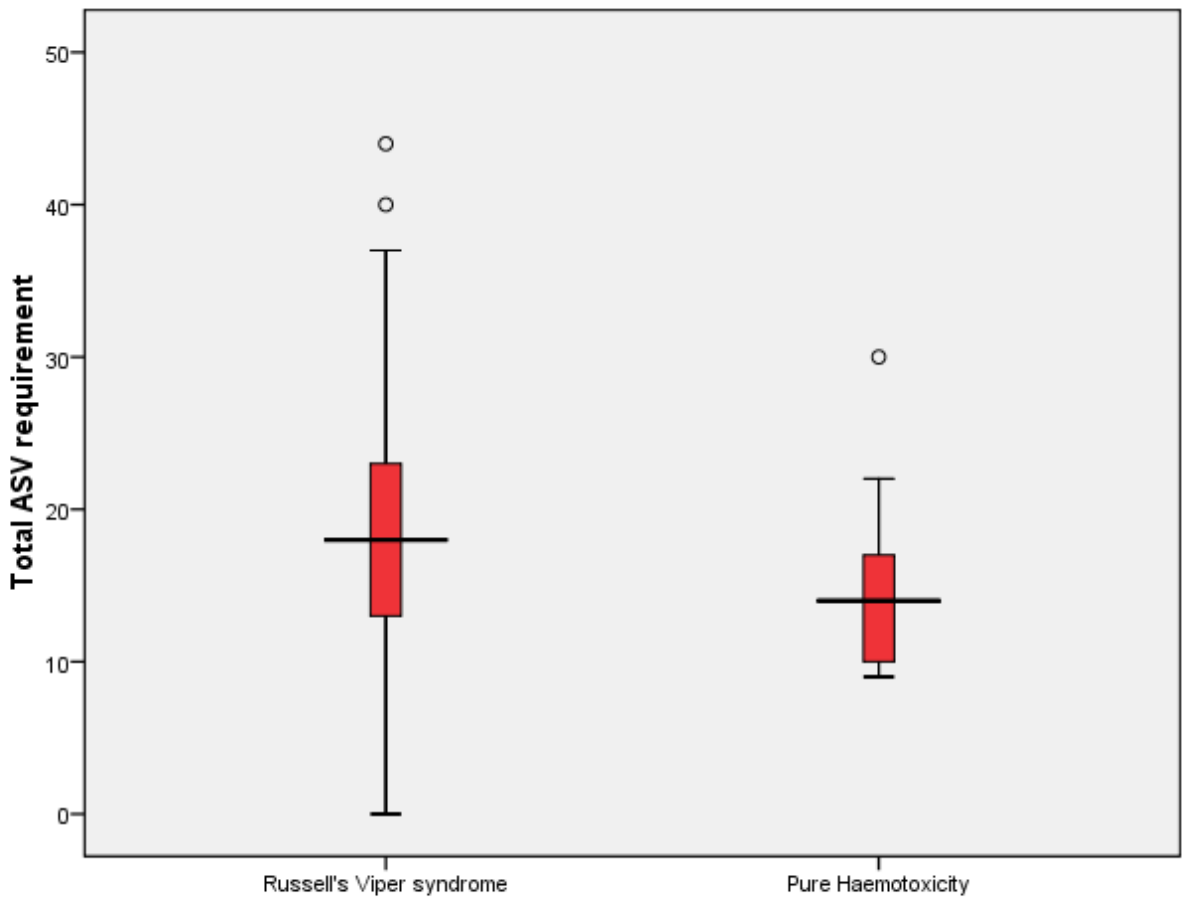


Figure 11 showing higher requirement of ASV in Russells viper envenomation when compared to pure haemotoxicity.

ICU stay and duration of mechanical ventilation

Most of the patients who presented to out center were referred cases and 40.11% (n=67) of them required ICU admission. The indications for ICU admission were mechanical ventilation, inotropic support requirement and renal replacement therapy/ plasmapheresis. The median duration of mechanical ventilation was 4 days (range1-21 days). The most common complication among the patients admitted in ICU was sepsis, most frequently due to ventilator associated pneumonia.

Unusual manifestations associated with snake bite

Myocarditis and cardiogenic shock

Myocarditis with cardiogenic shock has been described in the literature as a result of direct toxicity of the venom proteins. It has been associated with poorer outcome in view of the shock and associated with higher mortality rates.(22) 5.98% (n=10) of the patients had clinical signs of cardiac failure and evidence of cardiogenic shock with ECHO showing LV dysfunction and elevated cardiac enzyme levels suggestive of myocarditis. Myocarditis with LV dysfunction was most commonly seen associated with Russell's viper envenomation (7.84%, n=8). There was one case of transient myocarditis associated with pure haemotoxicity syndrome and one with cobra bite.

Cerebrovascular accident

There were 3 patients (1.79%) who developed cerebrovascular accident during their stay in hospital after snake bite. 2 cases had multiple infarcts in the cortex with permanent

residual neurological deficits and the outcome was poor. The third case developed a left cerebellar bleed with mass effect. This patient improved with medical management and did not require neurosurgical intervention.

Hypopituitrism

There were 2 (1.2%) cases of Hypopituitrism developing secondary to snake bite both of which had viperine manifestations associated (neurotoxicity and haemotoxicity). Hypopituitrism syndrome was confirmed by decreased hormonal levels suggestive of pituitary hypofunction in both the patients and MRI showing pituitary hemorrhage in one of them.

Other unsual manifestions of snake bite

There was one case of acute pancreatitis developing after snake bite associated with shock liver and another case of acute angle closure glaucoma post snake bite.

Outcome of patients (Figure 12)

There were 5 deaths with snake bite at our centre over the 2 years of the study with a mortality rate of 2.99%. Three patients were discharged against medical advice (1.8%).

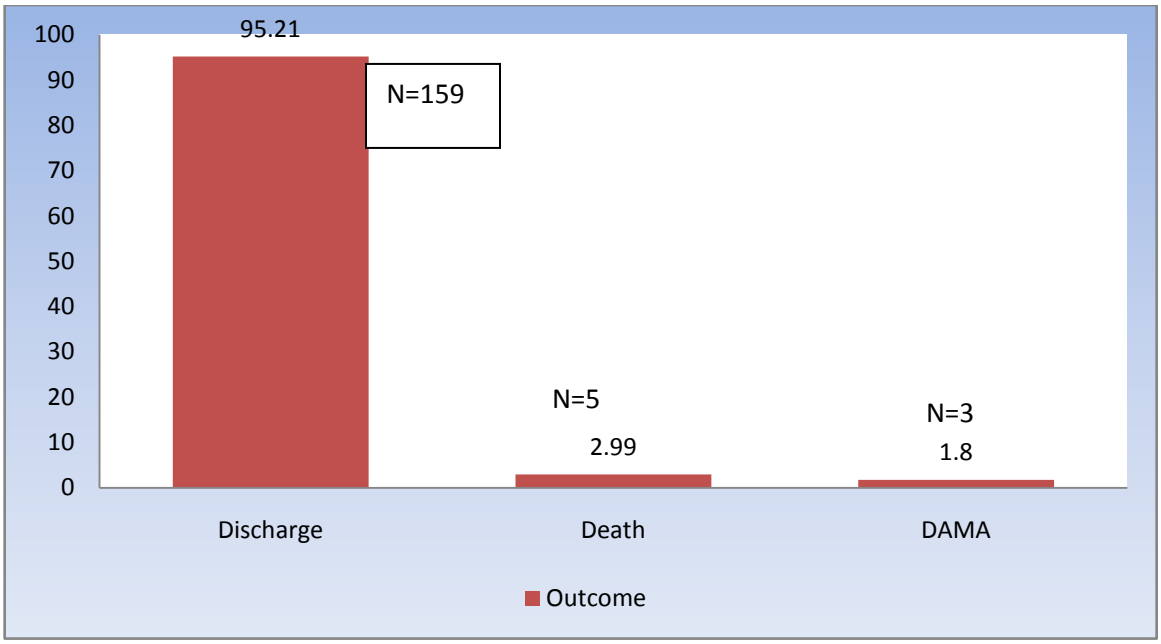


Figure 12- Outcome of patients with snake bite

Mortality analysis (Table 9)

There were 5 patients (2.99%) who died during their stay in the hospital. We observed that the combination of VICC, AKI, rhabdomyolysis and cellulitis were present in all of the patients who died. Table 2 highlights the common features which were present in the patients who expired in the hospital. Nearly all the patients died in the 2nd week due to late complications.

Table 9- Envenomation features associated with mortality

Patients	Haemotoxicity	Neurotoxicity	Cellulitis	Renal Injury	Rhabdomyolysis	Septic shock	Duration
A	+	+	+	+	+	+	7 days
B	+	-	+	+	+	+	12 days
C	+	-	+	+	+	-	11days
D	+	+	+	+	+	+	14 days
E	+	+	+	+	+	+	2 days

SECTION II- PLA2 LEVELS IN SNAKE ENVENOMATION

PLA2 levels were measured in 30 normal controls, 100 patients with snake envenomation and 64 snake bite patients with no envenomation.

Normal PLA2 activity in healthy controls

The mean PLA2 in 30 healthy controls was 62.57± 13.34 μmol/min/ml and the median PLA2 activity was 62.10 μmol/min/ml (41.9-91.1). This assay could not distinguish exogenous venom PLA2 and endogenous human PLA2,

Admission PLA2 levels and snake envenomation (Figure 11 and 12)

The median PLA2 activity at admission was 74.02 μmol/min/ml (40.2-180.6) for patients with systemic envenomation and 80.93 μmol/min/ml (40.9-151.6) in patients with snake bite without systemic envenomation and this difference was not significant (p=0.886). There were overlapping ranges with lower values of PLA2 found in both snake envenomation and non envenomated controls. The graph shows that higher values of PLA2 occurred only in systemic envenomation.

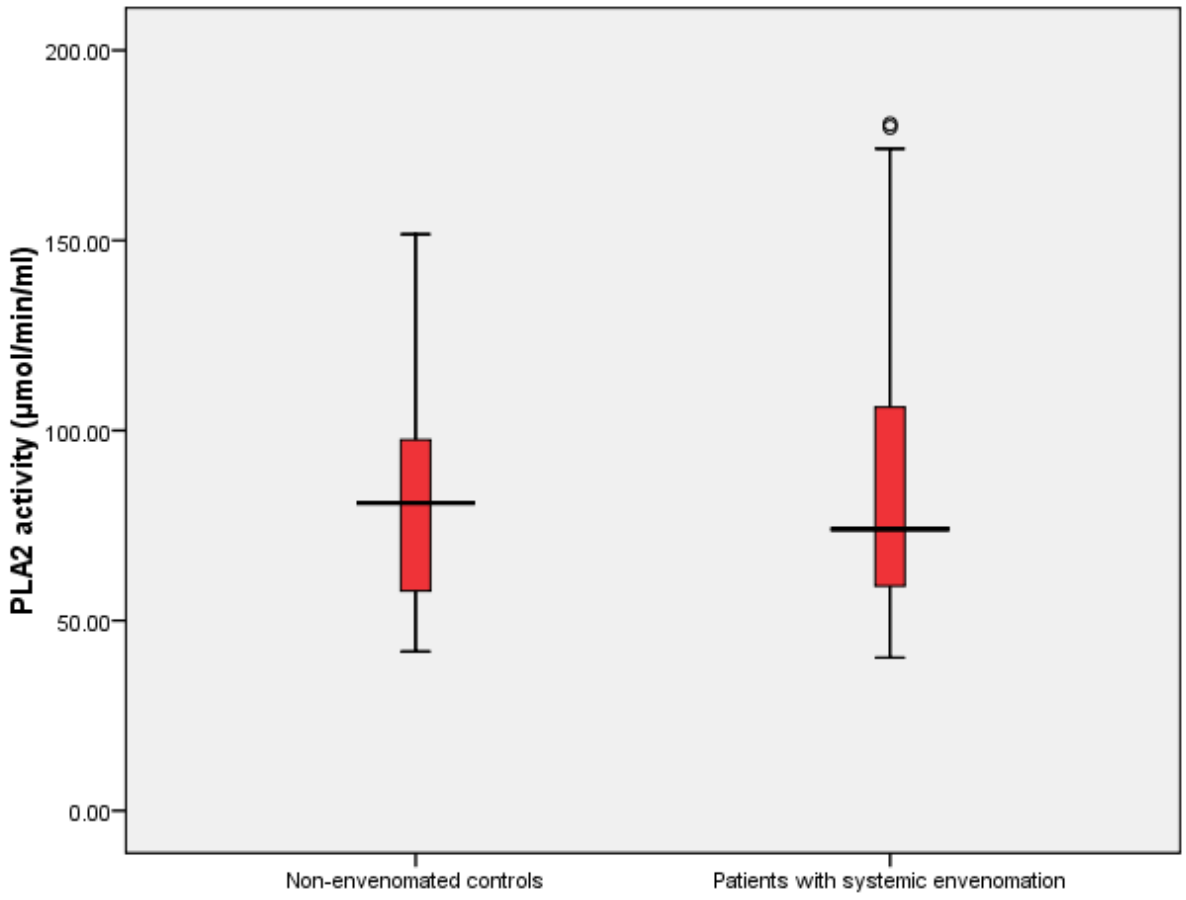


Figure 13 represents the box plot of the PLA2 values (median values with IQR) in systemic envenomation and patients with snake bite without any systemic envenomation.

The median PLA2 activity in patients with systemic envenomation, 74.02 µmol/min/ml (40.2-180.6) was significantly higher than normal controls 62.10 µmol/min/ml (41.9-91.1) (p=0.002).

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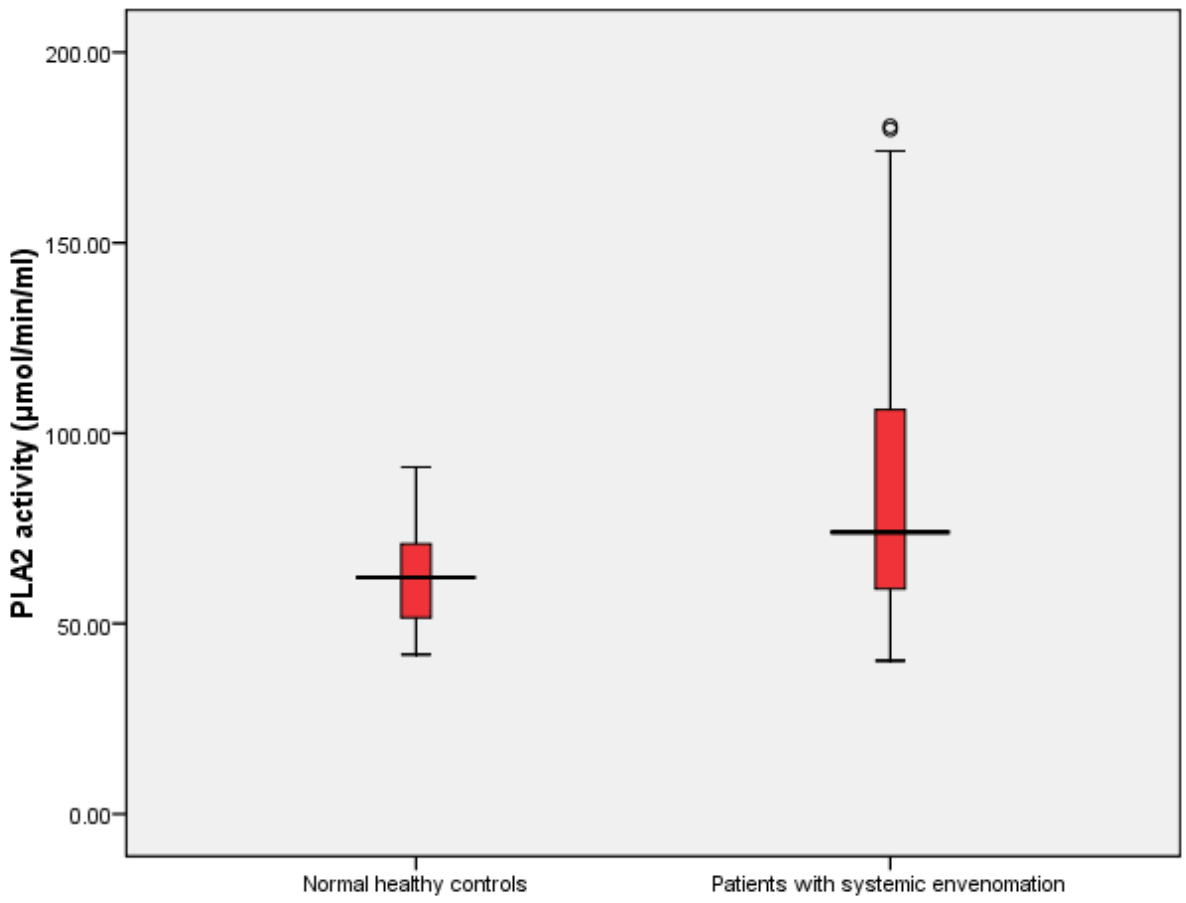

Figure 14 showing the box plot of PLA2 values highlighting the median with IQR for normal healthy controls and patients with systemic envenomation.

Relationship of PLA2 to specific snake bite related envenomation syndromes (Table 10)

This was done to assess whether PLA2 activity at admission could enable species specific diagnosis of snake envenomation in patients who developed systemic envenomation. Snake identification was limited to a few cases and hence it was decided to correlate PLA2 activity with species specific envenomation syndromes that were documented in envenomated patients.

Table 10 **PLA2 activity in different envenomation syndromes**

Envenomation syndrome	**Probable snake species implicated**	**No of subjects**	**Median PLA2 activity in µmol/min/ml(IQR)**
Pure haemotoxicity	Russell's/Saw scaled viper	16	74.77 (66.18-103.02)
Haemotoxicity+local reaction with neurotoxicity and/ or AKI	Russell's viper	63	79.86 (59.41-130.97)
All haemotoxic bites	Viperidae	79	79.61(63.6-120.1)
Neurotoxicity without local swelling	Krait	9	61.79 (53.1-65.3)
Neurotoxicity+local swelling	Cobra	12	60.8 (53.2-78.0)
Pure Neurotoxicity (without haemotoxicity)	Krait/Cobra	21	61.78 (53.22-70.39)

Comparison of PLA2 in different species specific envenomation syndromes (Figures 15-18)

1. Viperine envenomation with Elapidae bites (Krait /Cobra envenomation) (Figure 13

   It was noted that the admission median PLA2 values were higher for Viperine envenomation 79.61(IQR 63.6-120.1) μmol/min/ml when compared to Elapidae (Krait/Cobra) envenomation. 61.78 (IQR 53.22-70.39) μmol/min/ml and this difference is statistically significant (p=0.001).

The diagnostic utility of admission PLA2 in distinguishing between Viperine and Elapidae bites was examined using ROC curves (See Figure 14). The area under the curve was 0.743. A PLA2 level > 79.33 μmol/min/ml distinguished between Viperidae and Elapidae envenomation with a sensitivity of 50 % and specificity of 87.3%.

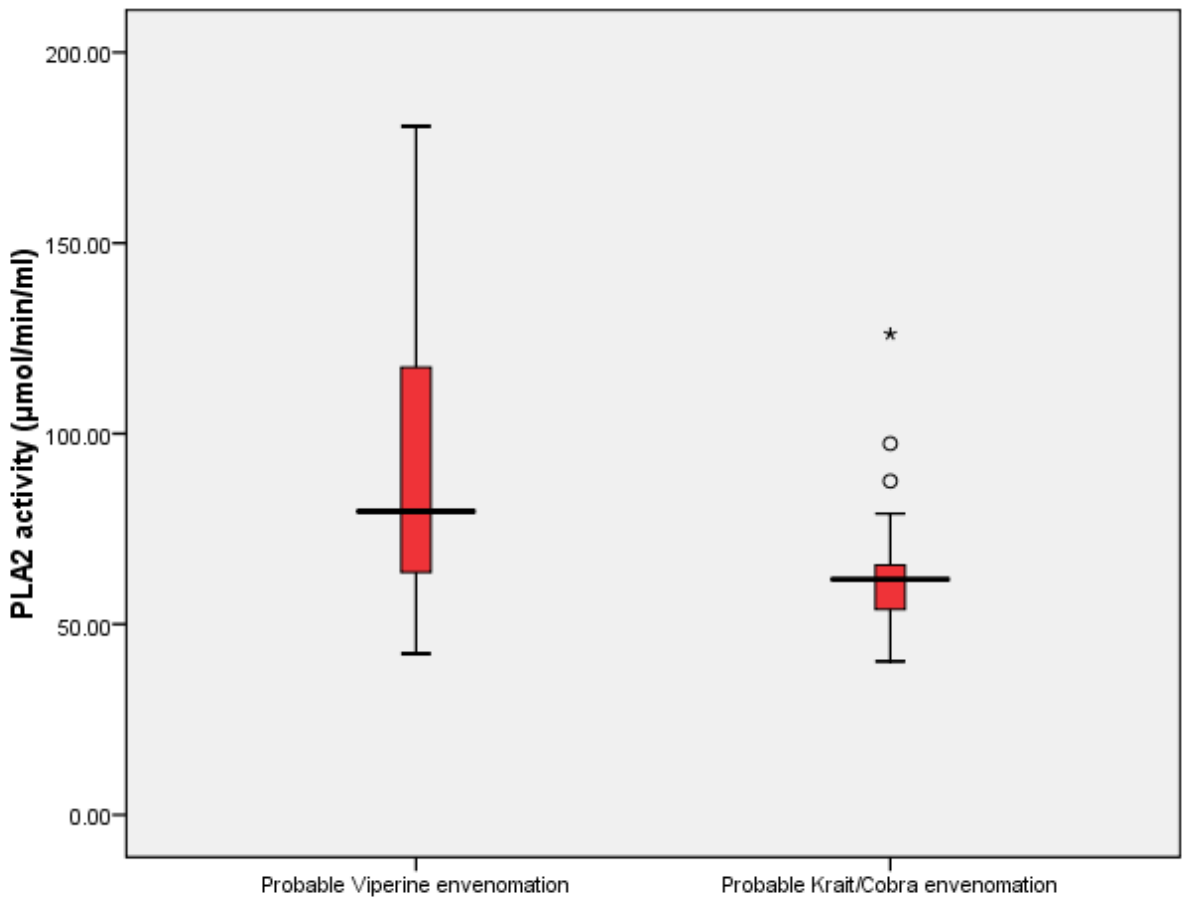

Figure 15 Comparison of admission PLA2 levels in Viper and Elapidae envenomationFigure demonstrating the box plot of PLA2 activity in Viperine versus Krait/Cobra envenomation.

ROC CURVE FOR PLA2 ACTIVITY TO DISTINGUISH VIPERIDAE AND ELAPIDAE ENVENOMATION

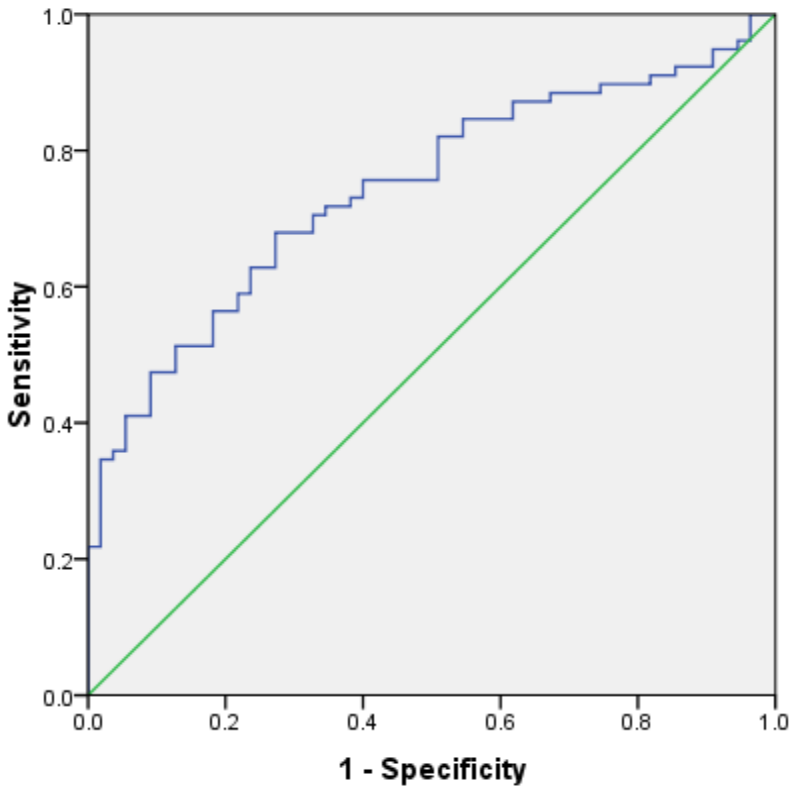

Figure 16 demonstrating the Receiver Operator Characteristic Curve for predicting Viperine envenomation when compared to Elapidae bites using PLA2 levels. The area under the curve is **0.743**. Values of PLA2 above 79.33 µmol/min/ml can distinguish Viperidae envenomation from Elapidae with a sensitivity of 50 % and specificity of 87.3%.

2. Comparison of pure hemotoxic envenomation syndrome and classical Russell's viper envenomation (Figure 17)

   The PLA2 levels in pure hemotoxic syndrome was 74.77 (66.18-103.02) µmol/min/ml and in Russell's viper syndrome was 79.86 (59.41-130.97) µmol/min/ml and there no statistically significant difference (p=0.971).

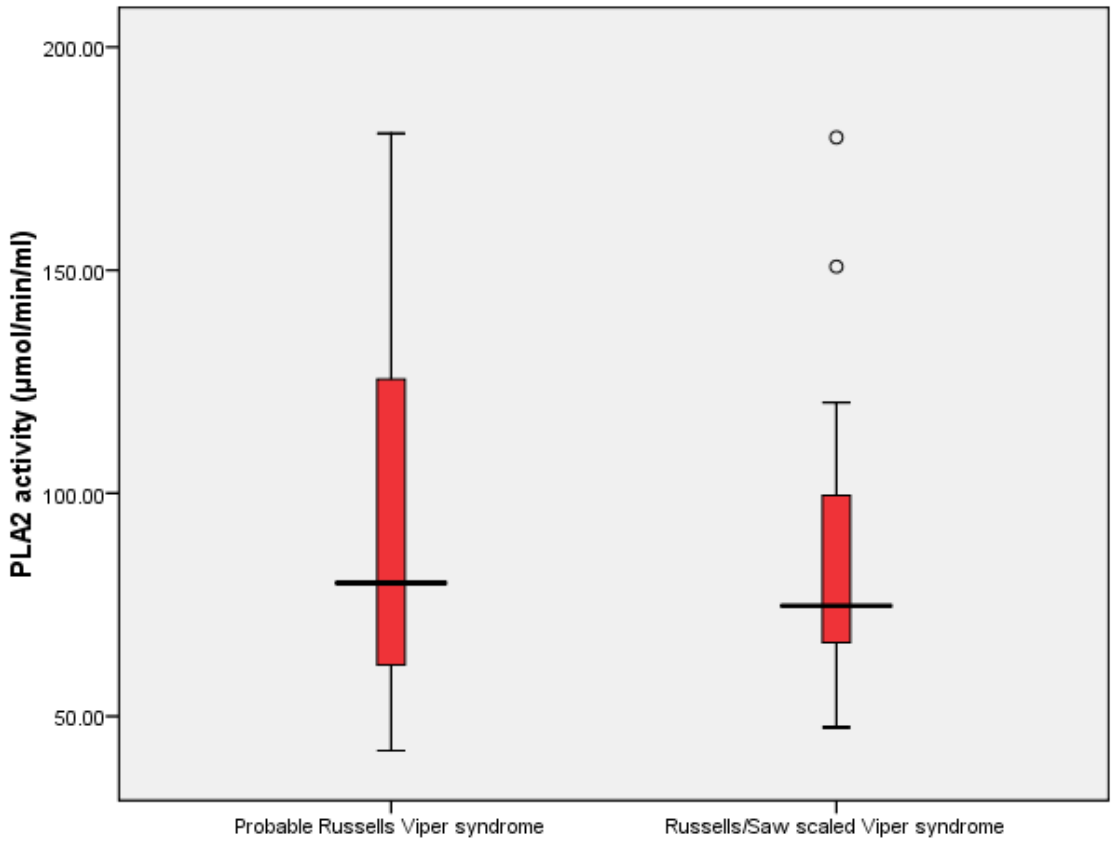

Figure 17 Comparison of admission PLA2 level in pure hemotoxic syndrome and classical Russell's viper syndrome

Figure showing the box plot of PLA2 activity in patients with Russell's viper envenomation with pure haemotoxicity.

3. PLA2 activity in Krait versus Cobra envenomation (Figure 18)

   The median PLA2 acitivity in Krait envenomation syndrome was 61.79 (IQR 53.1-65.3) µmol/min/ml and in Cobra envenomation syndrome was 60.8 (IQR 53.2-78.0) µmol/min/ml (p=0.602).

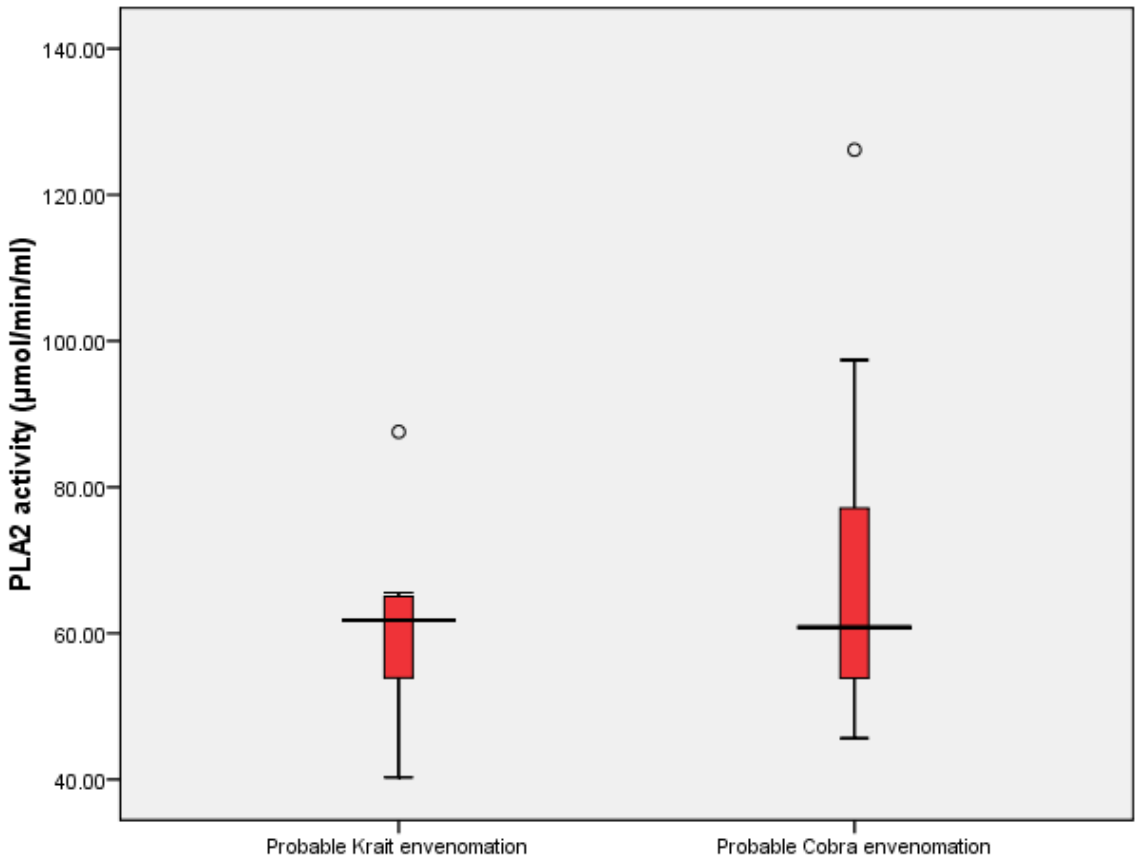

Figure 18 demonstrating the box plot of PLA2 activity with median and IQR in Krait and cobra envenomation.

Relationship of PLA2 to the severity of snake envenomation (Figure 19-22)

1. PLA2 acitivity and product support requirement in Viperine envenomation(All .haemotoxic bites) (Figure 17)

   The admission PLA2 activity was assessed for predicting the severity of haemotoxic envenomation in viperine bites. In patients with haemotoxicity whose coagulation parameters got reverted with ASV alone, the admission median PLA2 activity was 72.80 (IQR 59.4-90.8) μmol/min/ml. In patients who required ASV and product support for reversal of coagulopathy, the median admission PLA2 activity was 106.89 (69.7-144.8) μmol/min/ml. This difference was statistically significant (p=0.009).

   The prognostic utility of admission PLA2 in predicting severe viper envenomation requiring transfusion was evaluate using ROC curve (Figure 18). The area under the curve was 0.751. A PLA2 activity > 88.02 μmol/min/ml predicted requirement of product support with a sensitivity of 63% and a specificity of 84%.

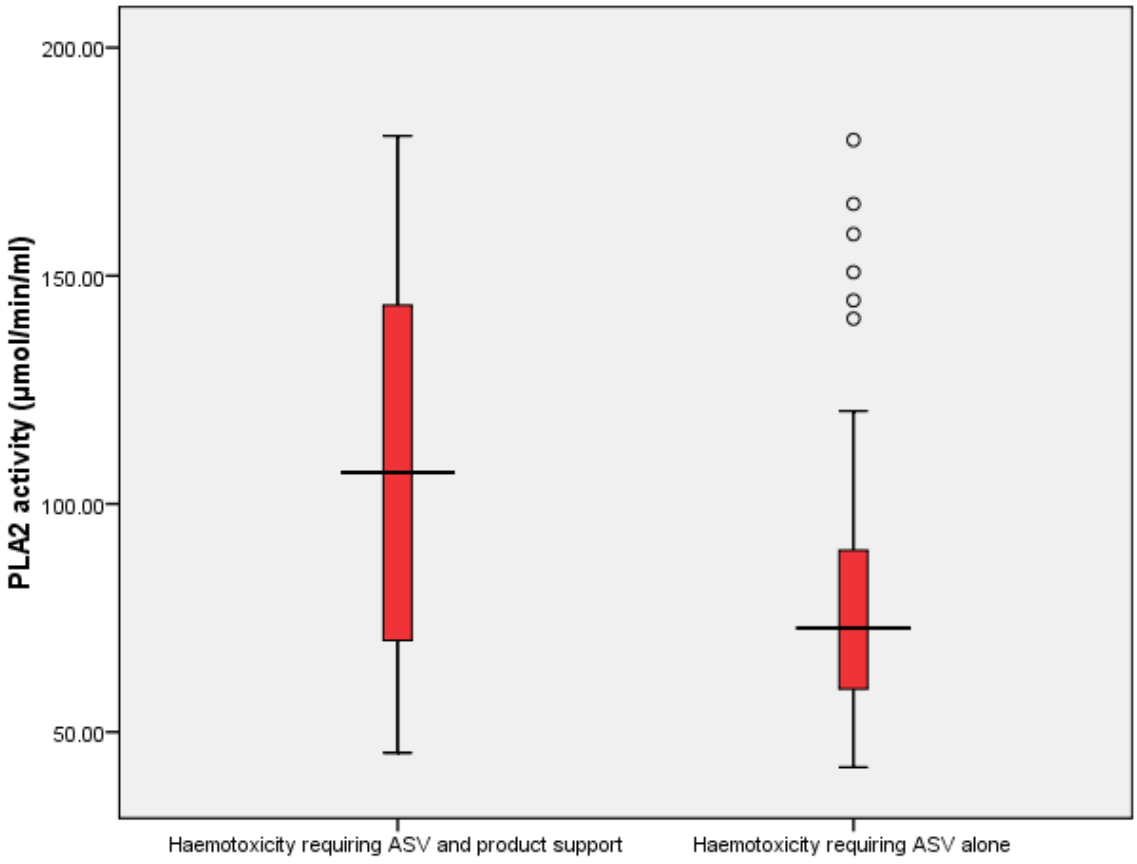

Figure 19 showing box plot of PLA2 activity with median and IQR in patients with severe haemotoxicity who required product support and those whose haemotoxicity got neutralised with ASV alone. It is noted that the admission PLA2 activity was higher in the patients who required product support.

ROC CURVE TO PREDICT PHOSPHOLIPASE A2 ACTIVITY TO PREDICT SEVERE HAEMOTOXICITY (PRODUCT SUPPORT REQUIREMENT)

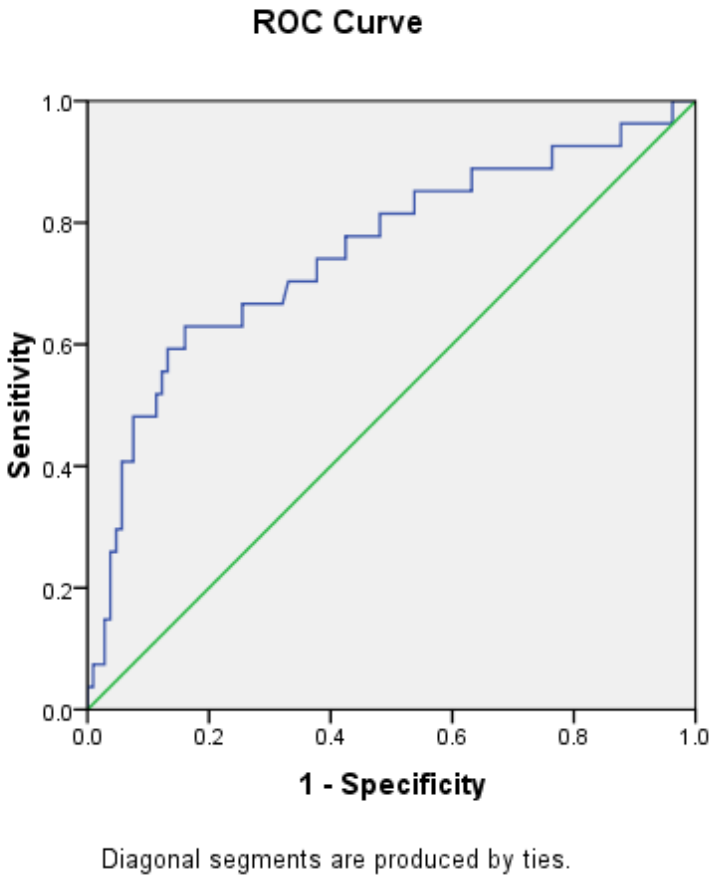

Figure 20 showing the ROC curve for PLA2 values to predict the product support requirement. Area under the curve is 0.751. PLA2 activity above 88.02 μmol/min/ml predicts requirement of product support with a sensitivity of 63% and a specificity of 84%.

2. PLA2 activity and viper envenomation with AKI requiring dialysis and no dialysis (Figure 21)

The admission PLA2 activity was assessed to predict the severity of acute kidney injury in Russell's viper envenomation. Patients who went on to require haemodialysis were considered to have severe envenomation when compared to patients whose renal parameters improved with ASV alone. The median PLA2 activity in patients with AKI who required haemodialysis was 106.89 (IQR 74.33-146.92) μmol/min/ml when compared to 70.36 (IQR 59.2-92.0)μmol/min/ml. This difference was statistically significant p=0.047.

The prognostic utility of admission PLA2 in predicting severe AKI requiring dialysis was analysed using the ROC curve (Figure 20). The area under the curve is was 0.752. An admission PLA2 level > 89.84 μmol/min/ml predicted requirement of haemodialysis with a sensitivity of 63.6% and a specificity of 83.8%.

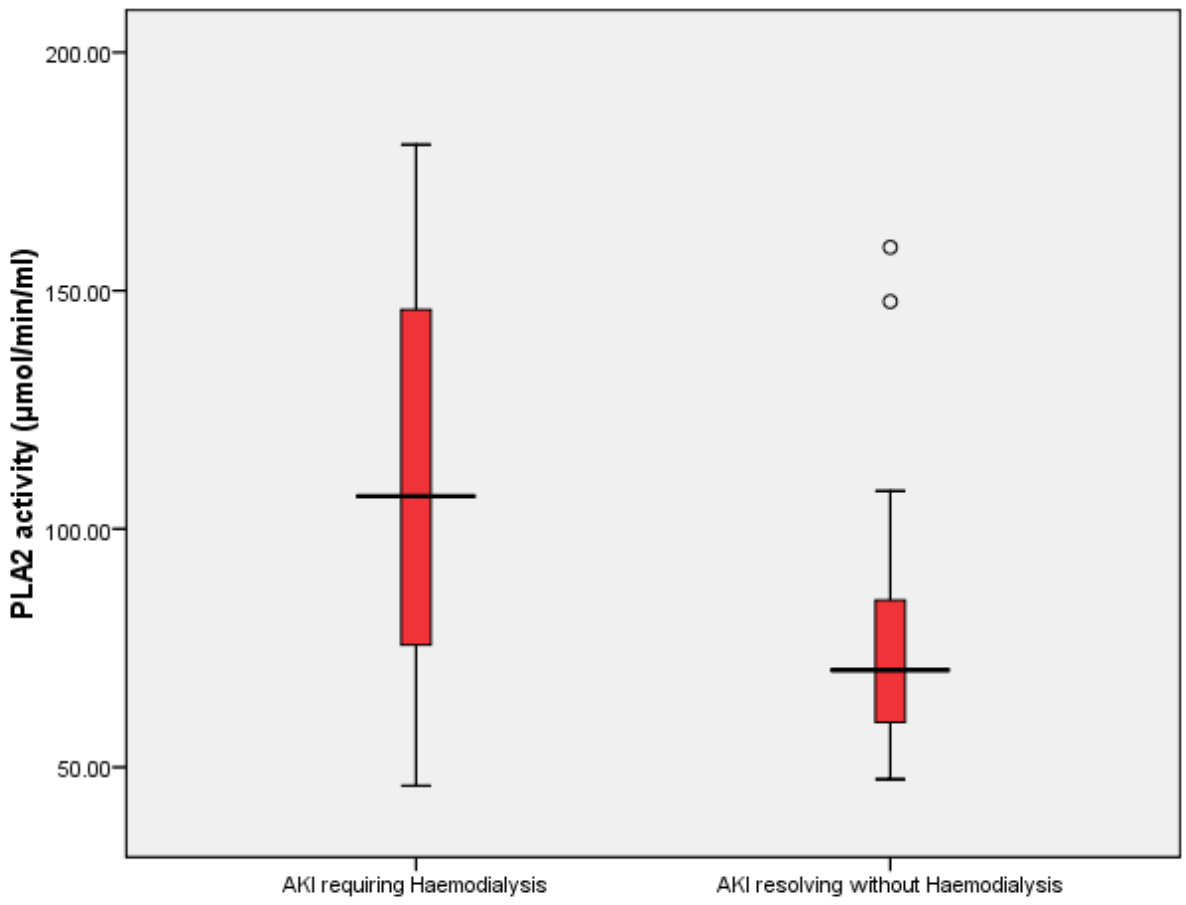

Figure 21 demonstrating the median PLA2 activity with IQR in patients with Russell's viper bite with AKI who required dialysis and who did not require dialysis. It is noted that the admission PLA2 values were significantly higher for patients who required haemodialysis.

ROC CURVE OF PLA2 ACTIVITY TO PREDICT AKI REQUIRING HAEMODIALYSIS

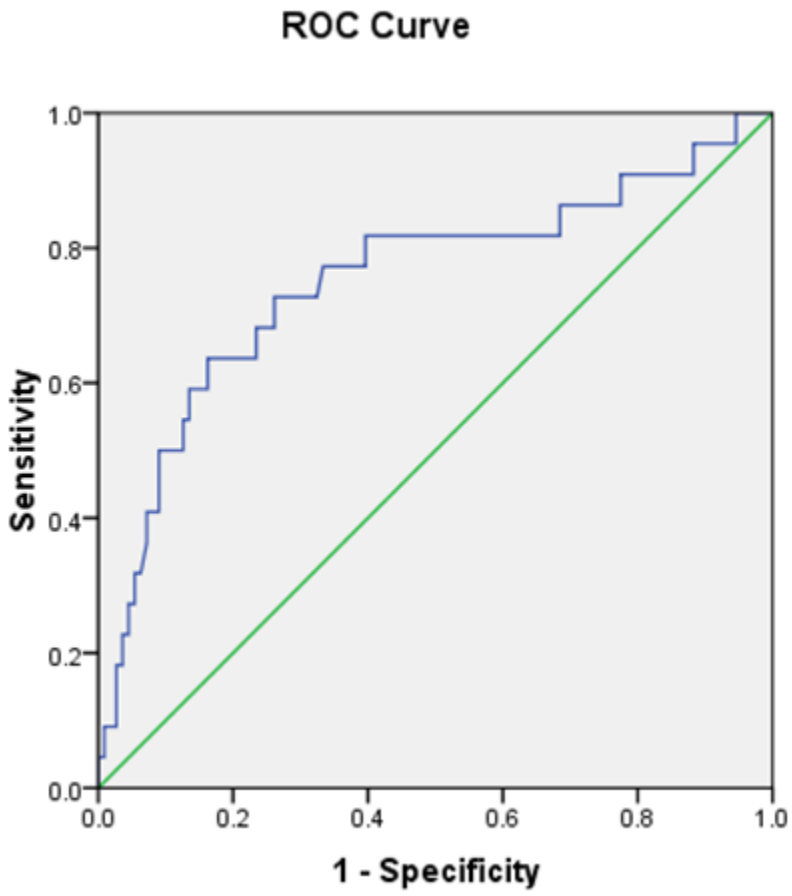

Figure 20 showing the ROC curve for the admission PLA2 values to predict the severity of AKI assessed as per the requirement of haemodialysis. The area under the curve is 0.752. PLA2 values above 89.84 μmol/min/ml predicts requirement of haemodialysis with a sensitivity of 63.6% and a specificity of 83.8%.

3. PLA2 level in pure neurotoxicity requiring mechanical ventilation and no mechanical ventilation (Figure 22)

The severity of neuroparalysis was assessed by the need for mechanical ventilation. The patients who required mechanical ventilation for respiratory muscle weakness had a median admission PLA2 activity of 63.48 (IQR 54.9-72.8) μmol/min/ml. The patients with neurotoxicity who did not require mechanical ventilation had a median admission PLA2 activity of 58.54(IQR 52.4-71.2) μmol/min/ml. This difference was not statistically significant (p=0.515).

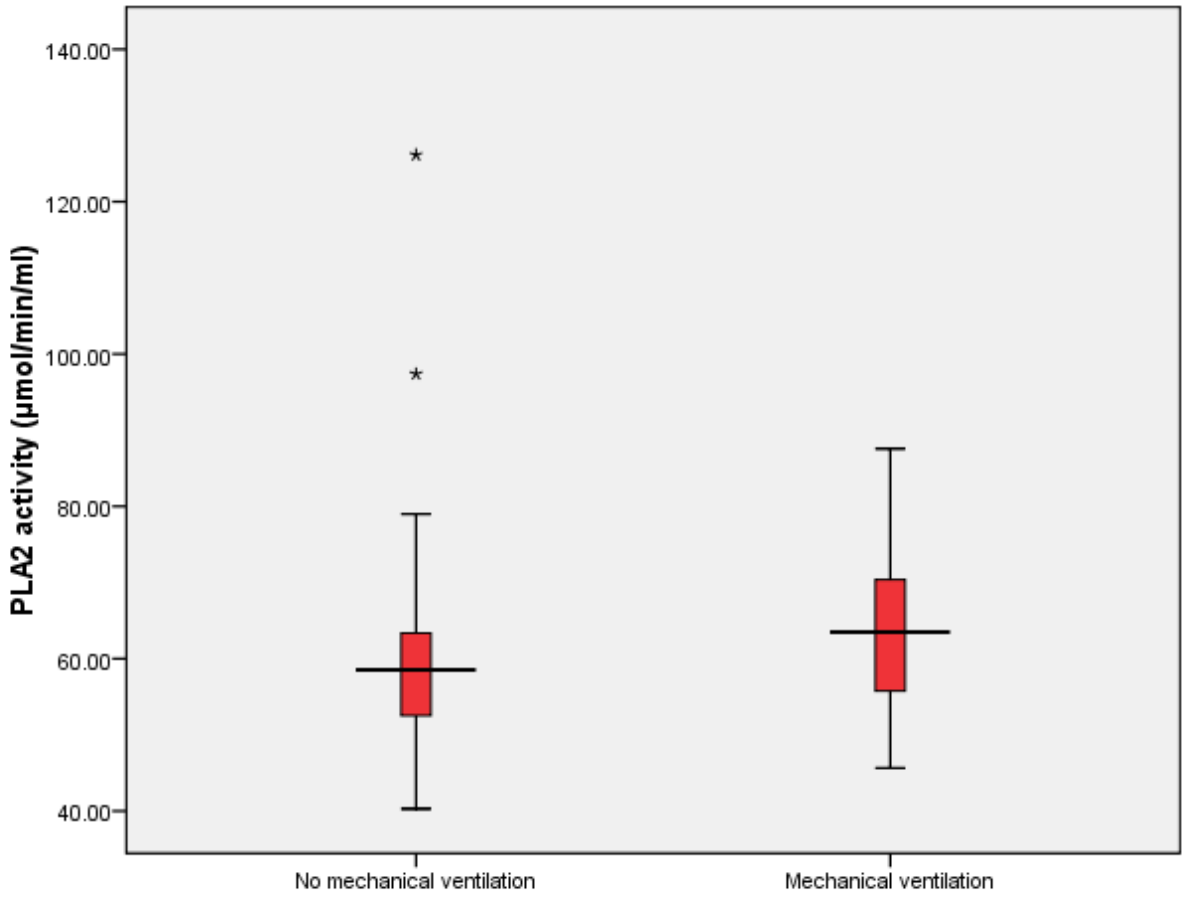

Figure 23 showing the box plot of PLA2 values in patients who required mechanical ventilation and those who did not require mechanical ventilation.

PLA2 activity and mortality

There was only one death among the patients in whom PLA2 was estimated. Hence meaningful conclusions could not be drawn between the co-relation of PLA2 activity with mortality.

Temporal profile of PLA2 activity in patients with systemic envenomation

There was significant declining trend in the mean PLA2 values from the time of admission till day 4 (p value=0.008). It is not clear whether the decline in PLA2 activity is due to decrease in venom PLA2 or endogenous PLA2.

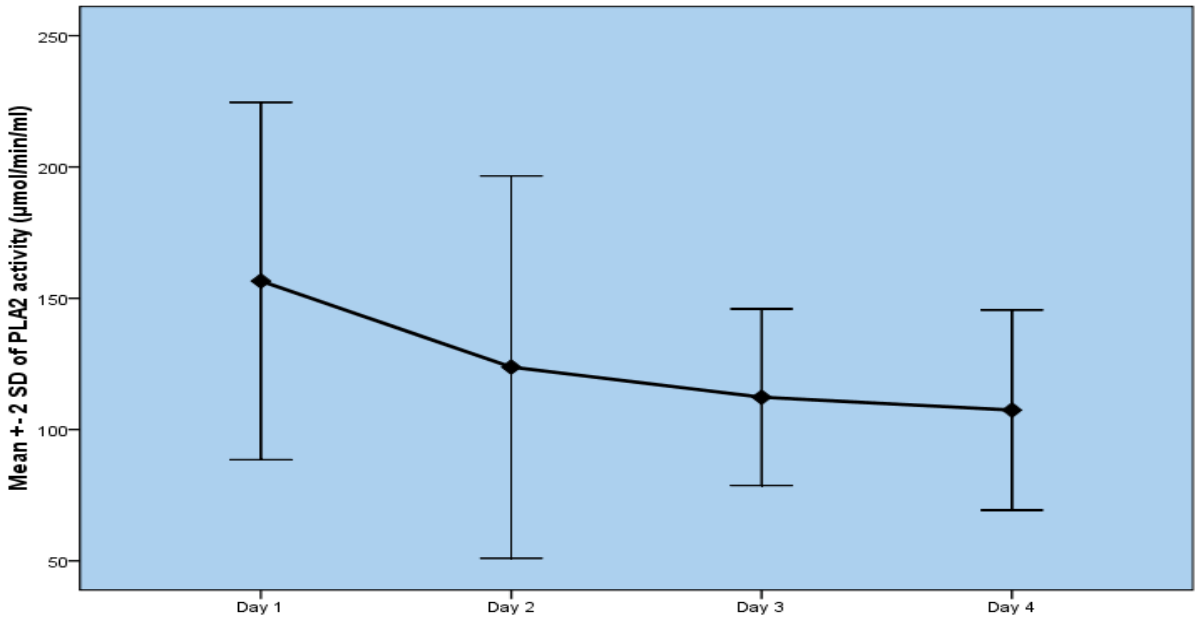

Figure 24 showing the decline in PLA2 values (represented diagrammatically as mean±2SD over time).

Discussion

The patients included in this study were referred from main government hospitals. Hence these patients were most severely envenomated patients of Vellore and adjacent districts. We have summarised discussion under 2 sections. 1. Clinical profile of snake envenomation. 2. PLA2 assay- diagnostic and prognostic utility

Clinical profile of snake envenomation

1. **Geographical variation in envenomation syndrome-Predominance of Russell's' viper envenomation and low rate of elapidae bite**

The most common clinical syndrome associated with systemic envenomation in our setting was haemotoxicity (Viperine envenomation). In haemotoxic patients, venom induced consumptive coagulopathy was the most common manifestation. The prevalence of a combination of local swelling, haemotoxicity with neurotoxicity and/or renal failure accounted for nearly two thirds of the patients with systemic envenomation. This suggests that Russell's viper envenomation is the most common cause of systemic envenomation in Tamil Nadu. This contrasts to studies from Western and Eastern India which show neurotoxicity as the most common envenomation syndrome. In this study the rate of Elapidae bites contributed to less than one fifth of the total systemic envenomation in Tamil Nadu. These results suggest that there is geographical variation in envenomation syndromes in Tamil Nadu compared to other parts of India.

2. Neuroparalysis spectrum in envenomation syndromes

The serious manifestations of neurotoxicity like bulbar weakness and limb weakness were short (1 day), but ptosis and ophthalmoplegia persisted for longer duration (3-5 days). The pattern of neuroparalysis did not show any species difference with regard to the incidence of ptosis, opththalmoplegia or bulbar weakness but the frequency of respiratory muscle weakness and limb weakness were higher in the Elapidae bites when compared to Russell's viper envenomation. The occurrence of neurotoxicity in Russell's viper envenomation was very high (85.3%, n=87). But the severity of neurotoxicity in Russell's viper envenomation was less when compared to Elapidae bites. Ptosis was the most common manifestation followed by ophthalmoplegia. The duration of various manifestations of neurotoxicity were short lived with lesser days for reversal with ASV in Cobra envenomation where as the duration of neurotoxicity in Krait/Russell's viper envenomation persisted for longer.

We report a higher incidence of neurotoxicity in Russell's viper envenomation (85.3%) when compared to Sri Lanka (59%). The neuroparalysis spectrum of Russell's viper in Tamil Nadu is similar to that of Sri Lanka with ptosis and ophthalmoplegia being the most common manifestation. The incidence of severe neurotoxic manifestations like respiratory muscle weakness and bulbar weakness in Russell's viper envenomation were relatively rare which is consistent with the results from Sri Lankan study.

3. **High rate of AKI and need for dialysis with Russell's viper bites**

The incidence of AKI in Russell's viper envenomation was 56.9% (n=58). One in three patients with Russell's viper envenomation required Haemodialysis 33.3% (n=34). This was much higher when compared to the incidence of AKI in Russell's viper bite in Sri Lankan study (19%).

The high rate in the present study could be due to referral bias as many patients may have been referred because of the presence of AKI.

4. **Significant myotoxicity with Russell's viper bites**

The incidence of muscle injury in Russell's viper bite was very high (45.05%). Among the patients with Russell's viper bite with AKI, nearly half (48.39%) had features of rhabdomyolysis. This is much higher when compared to the Sri Lankan study where the incidence of muscle injury was documented as 24% with Russell's viper envenomation.

These results suggest that muscle injury could be an important pathogenetic factor in development of AKI in Russell's viper envenomation.

5. **Cardiotoxicity with Russell's viper bites**

We report high rates of myocarditis associated with Russell's viper envenomation. 7.84% n=8 of the patients with Russell's viper envenomation had clinical/laboratory features of myocarditis and ECHO proven LV dysfunction. The cardiac effects of Russell's viper in Sri Lanka were documented as 3-12% . The literature of snake bite emphasised the

importance of Cobra bite induced myocarditis. However in this study nearly all the cases of myocarditis were due to Russell's viper bite.

6. Abdominal pain in Russell's viper bite

The incidence of abdominal pain in Russell's viper bite was estimated to be 15.53% which was much lower than the Sri Lankan data. In patients with Russell's viper envenomation with abdominal pain at presentation, 84.2% n=16 developed VICC, 78.9% N=15 developed neurotoxicity and 63.2% N=12 developed AKI which was similar to that of the Sri Lankan data. The literature in India has emphasised the association of abdominal pain with common Krait bite. However in this study abdominal pain was commonly associated with Russell's viper bite.

7. Pituitary insufficiency/Cerebrovascular accident with Russell's viper

There were 2 cases of acute pituitary insufficiency documented with Russell's viper envenomation (1.96%). It is a rare manifestation of Russell's viper envenomation similar to the data from Sri Lanka. Prospective studies from Myanmar show a higher rate of pituitary insufficiency.(68) Pituitary insufficiency may have been under diagnosed in this study as pituitary hypofunction may have been subclinical and required followup hormonal testing.

The incidence of non hemorrhagic cerebrovascular accident was 1.96% (n=2) among Russell's viper bite which was similar to the clinical data from Sri lanka(1.8%).

8. Mortality associated with Russell's viper envenomation

The mortality was exclusively associated with Russell's viper envenomation. 4.90% (n=5) of the patients with Russell's viper envenomation succumbed. The mortality was due to a combination of venom induced consumptive coagulopathy, AKI, rhabdomyolysis, local cellulitis in all patients and septic shock in the majority. The mortality due to Russell's viper envenomation is higher than Sri Lanka (2.6%).

9. Possible syndrome variation of Russell's viper bite in Tamil Nadu

When compared to data from Sri Lanka, the patients in this study had higher rate of neurotoxicity, AKI and muscle injury, greater requirement of blood transfusion and dialysis and greater mortality. This could suggest that the Russell's viper bite in Tamil Nadu causes greater organ damage and morbidity. These may represent variations in venom composition between Russell's viper in Tamil Nadu and Sri Lanka.

10. Clinical Antivenom inefficacy in Russell's viper envenomation

In this study the antivenom dose requirement was highest with Russell's viper envenomation syndrome. The ASV requirement in Viperine envenomation (most of which were Russells viper envenomation) was significantly higher than Elapidae bites. The greater antivenom requirement and the high rates of complications (product support requirement and haemodialysis) in Russell's viper bite probably indicate a relative inefficacy of polyvalent ASV to neutralize Russell's viper envenomation. The potential explanations for this inefficacy include: (1) lack of neutralisation of toxic venom proteins

by antivenom and (2) occurrence of organ injury in muscle and kidney prior to the initiation of antivenom. The inefficacy of ASV for Russell's viper envenomation has not been reported so far in the literature.

11. Allergy anaphylaxis rates

ASV reaction occurred in more than one third of the patients who were administered ASV. The common manifestations were mild like itching and urticaria which did not interfere much with the ASV protocol (same dose was administered over a longer time). The serious complications like bronchospasm and anaphylaxis were associated with poorer outcome and reduced use of ASV. This was similar to the incidence of ASV reactions in other geographical regions of South East Asian countries. (69)

Evaluation of Phospholipase A2 assay in diagnosis and assessment of severity of snake envenomation

The aims of this study were to assess the utility of serum PLA2 in (1) Diagnosis of snake envenomation (2) Assessment of severity of snake envenomation.

The main findings of this study were:

1. PLA2 levels were elevated in snake bite envenomation in comparison to normal controls
2. PLA2 levels were elevated in both envenomated and non envenomated snake bite and there was no significant difference.

3. PLA2 was elevated in viper bite syndromes compared to elapidae syndromes. PLA2 levels showed greater elevation in more severe viper bite syndromes requiring blood transfusion and dialysis.
4. PLA2 levels declined with time

Can serum PLA2 be used a test to diagnose snake envenomation?

The results suggest that while easy this assay is easy to perform it does not distinguish between envenomation and no envenomation in snake bite. Therefore this study shows that **serum PLA2 cannot be used as a diagnostic test for snake envenomation**. The elevation of serum PLA2 in viper bites compared to Elapidae bites suggest that it could be used to distinguish between haemotoxic and pure neurotoxic syndromes. A PLA2 level > 79.33 μmol/min/ml can distinguish Viperidae envenomation from Elapidae with a sensitivity of 50 % and specificity of 87.3%. However the clinical syndromes of Viper bite and Elapidae bites are clearly distinguishable and hence there may not be a role of a lab test to make this differentiation.

Can serum PLA2 be used to assess severity of envenomation?

PLA2 is elevated in more severe viper envenomation requiring transfusion and dialysis. The diagnostic test evaluations showed that PLA2 levels in viper bite predicted need for blood transfusion and dialysis with reasonable sensitivity and specificity. A PLA2 activity > 88.02 μmol/min/ml predicted requirement of product support with a sensitivity of 63% and a specificity of 84%. An admission PLA2 level > 89.84 μmol/min/ml

predicted requirement of haemodialysis in AKI with a sensitivity of 63.6% and a specificity of 83.8%.

However it is not clear if the elevation detected is endogenous due to inflammation and organ dysfunction or from the venom. Since the severity of renal injury and haemotoxicity in viper bite can be easily diagnosed through standard coagulation and renal tests, the role of PLA2 as a prognostic marker is limited.

Overall the study shows that PLA2 levels cannot be used to diagnose systemic envenomation though it may have some role in distinguishing between Viper and Elapidae bites and in predicting prognosis in Viper bites. The study highlights the limitation of using enzymes assays to diagnose snake envenomation. Although PLA2 is an enzyme which was described to be present in Viperine and Elapidae species of snakes in India, its role as a diagnostic and prognostic test may be limited in our setting. Further work is required to develop a diagnostic test for snake envenomation both to identify diagnose systemic envenomation and identify the species of snake.

Further research work

Russells's viper envenomation

Russell's viper envenomation is the important envenomation syndrome in Tamil Nadu. Detailed work is required to improve treatment for Russell's viper envenomation including:

(a) clinical spectrum and geographical syndrome variations in Russell's viper envenomation.

(b) Characterisation of venom proteins causing toxicity of Russell's viper envenomation in Tamil Nadu

(c) Further work on documenting efficacy of ASV for Russell's viper envenomation and improving quality of antivenom.

Diagnostic tests for snake bite

The present study showed the limitation of using enzymatic tests for diagnosis of snake envenomation. Therefore further work is required develop specific immunoassays for snake bite such has been developed in Australia. This involves identifying specific venom proteins of individual snakes (the big 4) and development of antibodies against these proteins that do not cross react with human antibodies.

Limitations of the study

1. The case distribution in the community may be different since most of the cases which were studied were referral cases with significant complications.
2. PLA2 measurements could be influence by delay in transfer, prior antivenom administration

Conclusion

1. Clinical study

Russell's viper envenomation is the most common cause of systemic envenomation in Tamil Nadu which is associated with high rates of neurotoxicity, muscle injury and AKI, need for blood transfusion and dialysis and significant mortality. This severe clinical envenomation syndrome could represent a syndrome variation of Russell's viper in Tamil Nadu in comparison to Sri Lanka. The higher dose of ASV and significant complication rate suggests that there is a relative inefficacy of ASV to neutralize the envenomation of Russell's viper when compared to other snake species. The study also documented the differences in the neuroparalysis spectrum of Russell's viper envenomation with lower rate of limb and respiratory muscle paralysis when compared to Cobra and Krait envenomation.

2. PLA2 as a diagnostic test for snake envenomation

PLA2 activity is not a useful test to diagnose snake bite patients with systemic envenomation although it may have a role in distinguishing between Viper and Elapidae bites and assessing prognosis of Viper bites.

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ANNEXURES

PATIENT INFORMATION SHEET

STUDY TITLE

SNAKE VENOM PROTEINS AND ENVENOMATION SYNDROMES

INFORMATION TO PATIENT

Snake bite is a common emergency. There are four major snake species causing envenomation, Cobra, Russel's viper, Saw scaled viper and the common Krait. However it is known that there are many other snake species that are venomous. There is need for studies to correlate snake bite symptoms and signs to snake venom proteins. This study aims to correlate the envenomation syndromes with the venom proteins that are detected in the serum of patients. We will be obtaining blood samples for further studies examining venom proteins as part of separate study. This study is an observational study and will not influence your treatment. The best possible treatments will be given to you as recommended.

Recruitment is purely voluntary and at no cost to you/your relative. The results of the blood test will be kept confidential. You/your relative may choose to withdraw from this study at any time. The care provided to you/your relative will not be affected by your decision to participate or not in this study. However, there is no direct benefit to you/your relative from this study.

CONSENT SHEET

I_____ , daughter / son of _____ , am aware that I am being asked to take part in the study, " Snake venom proteins and envenomation syndromes"

The data collected for this study can be used for publication purposes. As part of this research, history and examination will be obtained and data from my chart and daily patient notes will be recorded in a proforma. In addition, I asked to provide a small amount of my blood (about 5 ml), every day for 5 days. If a dead snake specimen is brought by me, it will be stored and used for further studies.

- I have read and understood the information sheet for this study and have had the opportunity to ask questions.

- My participation in the study is voluntary and that I am free to withdraw from this study at any time without giving any reason, without my medical care or legal rights being affected.

- The blood samples will be used for future studies of detection of snake venom proteins towards development of venom detection tests and understanding mechanism of toxicity.

- My identity will not be revealed in any information released to third parties or published.

- There will be no restriction on the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I agree to take part in the above study.

Signature of participant:

Date:

Thumb impression if patient cannot sign

Signatory's Name:

Date:

Study Investigator's Name:

Name / Signature of the Witness:

INFORMED ASSENT FORM

(Instruction: This form is to be used if patient is a minor or too sick to obtain informed consent. The assent should be obtained from a close relative.)

I _____, _____, _____

(Name of relative) (Relationship) (Patient name)

am aware that my relative is being asked to take part in the study, " Snake venom proteins and envenomation syndromes"

The data collected for this study can be used for publication purposes. As part of this research, history and examination will be obtained and data from my relative's chart and daily patient notes will be recorded in a proforma. In addition, my relative is being asked to provide a small amount of my blood (about 5 ml) every day for a total duration of 5 days.

- I have read and understood the information sheet for this study and have had the opportunity to ask questions.
- My relative's participation in the study is voluntary and that he/she is free to withdraw from this study at any time without giving any reason, without his/her medical care or legal rights being affected.

.

- The blood samples will be used for future studies of detection of snake venom proteins towards development of venom detection tests and understanding mechanism of toxicity.
- My relative's identity will not be revealed in any information released to third parties or published.
- There will be no restriction on the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

On behalf of my relative, I am agreeing to his/her participating in the above study.

Signature of relative:

Date:

Thumb impression if relative cannot sign

Signatory's Name:

Relationship:

Study Investigator's Name:

Name / Signature of the Witness:

STUDY TITLE:

Snake venom proteins and envenomation syndromes

PATIENT INFORMATION SHEET

Snake bite is a common emergency. There are four major snake species causing envenomation, Cobra, Russel's viper, Saw scaled viper and the common Krait. However it is known that there are many other snake species that are venomous. There is need for studies to correlate snake bite symptoms and signs to identification of snakes. Since the number of dead snakes brought is very small, we need diagnostic tests to identify snake species. This study aims to correlate the clinical profile of the patients with the venom proteins that are detected in the serum. We will also be obtaining blood samples for further studies examining venom proteins as part of separate study. This study is an observational study and will not influence your treatment. The best possible treatments will be given to you as recommended.

In this study you will be provided standard of care treatment including anti-venom, ICU care, antibiotics and other treatments as required. This study is an observational study and does not include any treatment. Hence it will not influence the quality of the care you receive or the outcome of treatment.

At admission the doctors looking after you will perform a history, examination and appropriate laboratory tests. They will obtain a 5 ml sample of blood, urine and swab from the bite site at admission and a follow up blood samples of 5 ml taken on a daily basis for 5 days.

These samples will be transported to the Christian Medical College for laboratory studies in the Neurochemistry laboratory. The tests performed at Christian Medical College are on the bite swab specimen. The bite site swab specimen will be studied for DNA from the snake using a technique called polymerase chain reaction (PCR). This technique involves increasing the number of copies of DNA specific to the snake. The DNA is then analyzed by a technique known as DNA sequencing to know the information of the base pairs that comprise the DNA specific to the snake. This will be compared to the database of snake sequences across the world to identify the snake that has bitten the patient.

Since we are doing such a detailed study, we would like to obtain samples of urine and blood for identifying the proteins form the venom. This subsequent study will be towards developing a venom detection test.

During your hospitalization, you will be examined every day till discharge. The information regarding your name and address will be kept confidential and will not be revealed to another person outside the investigators of the study.

For any queries you can contact, Dr George Abraham, PG Registrar,Department Of Medicine, CMC Vellore,Ph-9486136172

National Snakebite Study (modified) **Study Number** ☐☐ ◇ ☐☐☐☐☐

OP Number: _____ **IP Number:** _____ **Date Enrolled:** _____

I. Patient Profile:

Patient identification:

Name: _____ Age:_____ Sex: **M** ☐ **/ F** ☐

Village: _____ District: _____ State: _____

Date of Bite: _____ Date of admission: _____

Time of bite: _____ Time of admission:_____

Site of bite: RUL ☐ RLL ☐ LUL ☐ LLL ☐ Face ☐ Trunk ☐ Other ☐ Bite site Unknown ☐

Local swelling: **Y** ☐ **/ N** ☐ Blisters: **Y** ☐ **/ N** ☐ Local Bleeding: **Y** ☐ **/ N** ☐

Place where bitten: Indoor ☐ Outdoor ☐ Unknown ☐

Activity of the person during the bite: Awake ☐ Asleep ☐ Unknown ☐

II. Identification of the snake:

Was the snake brought? : **Y** ☐ **/ N** ☐

III First Aid Profile:

- Tourniquet: **Y** ☐ **/ N** ☐ Bite site Washed: **Y** ☐ **/ N** ☐

Previous Hospital Treatment: Y ☐ **/ N** ☐

If yes, proceed below:

- Type of the hospital: PHC ☐ CHC ☐ District Govt. hospital ☐ Private hospital ☐
- Tourniquet: **Y** ☐ **/ N** ☐ Time of removal of Tourniquet/Compression Bandage: _____
- ASV administered outside: **Y** ☐ **/ N** ☐ If yes, number of vials: _____
- History of allergy to ASV: Itching ☐ Urticaria ☐ Angiooedema (Swelling of Mouth & tongue) ☐ Bronchospasm ☐ Anaphylaxis (Shock) ☐

IV. Study sample:

- Bite Site Swab Obtained: **Y** ☐ **/ N** ☐
- Blood Sample Obtained: **Y** ☐ **/ N** ☐
- Urine Sample: Obtained: **Y** ☐ **/ N** ☐
- Before ASV: **Y** ☐ **/ N** ☐
- Follow up blood sample at 12Hrs obtained: **Y** ☐ **/ N** ☐

V. Clinical Profile at admission:

Clinical Examination at Admission:	**Bleeding Manifestations:**
BP < 90/60: **Y** ☐ / **N** ☐	Oral Mucosal Bleeding: **Y** ☐ / **N** ☐
Respiratory rate > 24/Min: **Y** ☐ / **N** ☐	Hemoptysis: **Y** ☐ / **N** ☐
Altered Sensorium: **Y** ☐ / **N** ☐	Hemetemesis: **Y** ☐ / **N** ☐
If yes GCS: _____	Melaena: **Y** ☐ / **N** ☐
	Hematuria: **Y** ☐ / **N** ☐
	Bleeding from other sites: **Y** ☐ / **N** ☐
Neurological Manifestations:	**Renal Failure:**
Higher mental functions-Handedness-----	Oliguria < 500ml/Hr: **Y** ☐ / **N** ☐
Intelligence---,consciousness-----, Memory-----,	
Orientation------, Emotion------Speech---------	**Abdominal findings**
Cranial nerves	Abdominal pain: **Y** ☐ / **N** ☐
Visual acuity-	Tone of the limbs-
Papilledema	Deep tendon reflexes-
Colour vision	Sensory Nervous system-Touch—
Confrontation test	Pain---
Drooping of Eye Lids: **Y** ☐ / **N** ☐	Temeprature-----
Ophthalmoplegia: **Y** ☐ / **N** ☐	Vibration----
Extraocular movements-	Position sense------
Bulbar weakness-	Superficial reflexes-
Neck Muscle weakness: **Y** ☐ / **N** ☐	Babinski s sign-
Muscle weakness of limbs: **Y** ☐ / **N** ☐	Rombergs sign-
If weakness is present	Cerebellar signs-
Grade of Power	Meningeal signs
RUL _____ LUL----------	Skull/spine-
RLL: _____ LLL---------	
Single breath count-	

VI. Laboratory Profile at admission:

Platelets:	**Urine microscopy:**	**20 min WBCT:**	**Blood urea:**	**Serum Creatinine:**	**Clotting Time:**
_____	Albumin Present ☐ Absent ☐ Hematuria(More than 5 RBC) Present ☐ Absent ☐	Clotted within 20 min ☐ Incoagulable at 20 min ☐	_____	_____	_____

Prothrombin Time (PT)		**Partial Thromboplastin (aPTT)**	
Patient (sec)	**Control (sec)**	**Patient (sec)**	**Control (sec)**
_____	_____	_____	_____

Outcomes: (For patients with no clinical signs of envenomation for 24 hours)

- Brought Dead ☐ Discharged ☐ DAMA ☐ Referred to higher centre ☐
- Date of Discharge ☐ Death ☐ DAMA ☐ Referral ☐ : _____
- Time of Discharge ☐ Time of Death ☐ : _____
- If referred, reason for the referral :

1. On Patient Request ☐
2. Need for Mechanical Ventilation ☐
3. Need for Dialysis ☐
4. Clinical Bleeding ☐
5. Others ☐

National Snakebite Study (modified) **Study Number** ☐☐◇☐☐☐☐☐

Daily Monitoring Proforma:

	Day 1	Day 2	Day 3	Day 4	Day 5
Bleeding	Y☐ / N☐	Y ☐/ N ☐	Y ☐ N ☐	Y ☐/ N ☐	Y ☐ N ☐
Paralysis	Y☐ / N☐	Y ☐/ N ☐	Y ☐ N ☐	Y ☐/ N ☐	Y ☐ N☐
Local swelling	Y☐ / N☐	Y ☐/ N ☐	Y ☐ N ☐	Y ☐/ N ☐	Y☐ N☐
Cardiotoxicity-BP **Abnormal CVS Examination** **ECHO(if done)**					
Platelets					
Serum Creatinine					
Urea					
PT/APTT					
Coagulopathy **Fibrinogen**	Y☐ / N☐	Y☐/ N☐	Y☐/ N☐	Y☐/ N☐	Y☐/ N☐
Ptosis	Y☐ / N☐	Y☐/ N☐	Y☐/ N☐	Y☐/ N☐	Y☐/ N☐
Ophthalmoplegia	Y☐ / N☐	Y☐/ N☐	Y☐/ N☐	Y☐/ N☐	Y☐/ N☐
Single breath count					
Grade of power **RUL/LUL/RLL/LLL**					
Rhabdomyolysis **LDH/CPK**	Y☐ / N☐	Y☐/ N☐	Y☐/ N☐	Y☐/ N☐	Y☐/ N☐
Rare complications like CVA, blindness,ARDS, any other syndromes not described above					

Treatment Data:

Antivenom: Y☐ / N☐ If yes, proceed to the following:

Lyophilized ☐ Liquid☐ Manufacturer name: _____ Batch: _____

Indication for ASV: Local Swelling☐ Coagulopathy☐ Shock☐ Neurological Symptoms☐ Unclear ☐

ASV schedule:

Dose	Date	Time	Number of vials
1st Dose			
2nd Dose			
3rd Dose			
4th Dose			
5th Dose			

Allergic reactions to ASV: **Y☐ / N☐** If yes proceed to the following:

Itching ☐ Urticaria☐ Angiooedema (Swelling of mouth & tongue) ☐Bronchospasm ☐Anaphylaxis (Shock) ☐

Treatment of Allergic reactions: Antihistamine☐ Hydrocortisone☐ Adrenaline☐ SC☐ IM☐ IV☐

Preventive treatment for allergic reactions: Antihistamine☐ Hydrocortisone☐ Adrenaline☐ SC☐ IM☐ IV☐

Blood transfusion: **Y☐ / N☐**

If yes: Whole Blood☐ FFP☐ Platelets☐ Number of transfusions: _____

Mechanical ventilation: **Y☐ / N☐** Duration: _____ (Days)

Tracheostomy done **Y☐ / N☐**
Ventilator associated pneumonia **Y☐ / N☐**

Neostigmine: Y☐ / N☐

Dialysis: **Y** ☐ **/ N** ☐

If yes: Haemodialysis ☐ Peritoneal dialysis ☐ Number of sessions of dialysis: _____

Antibiotics given: **Y** ☐ **/ N** ☐

Antibiotics: _____

Surgical intervention: **Y** ☐ **/ N** ☐

If yes: Debridement ☐ Fasciotomy ☐ Amputation ☐ Others ☐

Need for Surgery:

Final Case Summary:

Recovered ☐ Died ☐ DAMA ☐ Referred to higher centre ☐

Date of Discharge ☐ Death ☐ DAMA ☐ Referral ☐ : _____

If referred, reason for the referral: _____

If patient died, fill Proforma 4 (Death proforma)

Subgroups: 0

Number to be filled at the time of discharge: ☐

1. No envenomation ☐
2. Local swelling only ☐
3. Haemotoxicity with local swelling ☐

4. Haemotoxicity without local swelling ☐
5. Neurotoxicity only ☐
6. Neurotoxicity with Local swelling ☐
7. Haemotoxicity + Neurotoxicity with local swelling ☐
8. Haemotoxicity + Neurotoxicity without local swelling ☐
9. Haemotoxicity + Neurotoxicity + Renal failure with local swelling ☐
10. Haemotoxicity + Neurotoxicity + Renal failure without local swelling ☐
11. Haemotoxicity + Renal failure with local swelling ☐
12. Haemotoxicity + Renal failure without local swelling ☐
13. Others ☐ Explain: _____

Consultant/Doctor:

Name and designation: _____

Signature: _____ Date: _____

Death Proforma:

Date of Death: _____

Time of death: _____

Cause of death (tick appropriate):

1. Due to snake bite complications
   a. Respiratory failure ☐
   b. Bleeding ☐ Indicate site of bleeding: _____
   c. Renal failure ☐
   d. Cellulitis ☐
   e. Cardiac Arrhythmias ☐ Cardiac Failure ☐
   f. ARDS ☐

2. Anaphylactic shock ☐
3. Treatment associated death
   a. Ventilator associated pneumonia ☐
   b. Dialysis associated problems ☐
   c. Tube dislocation ☐
   d. Ventilator Malfunction ☐
   e. Other nosocomial infections ☐
   f. Others (Unspecified) ☐
4. Any other factors contributing to death (unrelated to snakebite or treatment)

Place of Death (tick appropriate):

Emergency ward ☐

Ward ☐

ICU ☐

During transfer ☐

After referral ☐

Postmortem performed: **Y**☐ **/ N**☐

Postmortem findings:

a)

b)

c)

d)

Study coordinator is to inform CMC on date of death. Death proforma and copy of death certificate to be send within 1 week.

Data Sheet

Study.I	Age	Sex	Site of bite	Date of Bite	Prior AS	Allergy	Itching	Urticari	Angioo	Bronch	Anaphy	Total A	Time G	Snake b	Snake S	Neurot	Ptosis	Opthalr	Bulbar	Respira
1	25	M	LUL	2/13/2014	0	0	0	0	0	0	0	0	8	Y	Russels	0	0	0		
2	54	M	LUL	2/16/2014	0	0	0	0	0	0	0	0	1	N		0	0	0		
3	35	M	LUL	2/24/2014	2	1	1	1	0	0	0	2	n	Y	Saw sca	1	1	0		
4	39	F	LUL	3/5/2014	0	0	0	0	0	0	0	20	2	N		0	0	0		
5	38	M	RLL	3/5/2014	0	0	0	0	0	0	0	12	4	N		0	0	0		
6	50	M	RLL	3/17/2014	10	0	0	0	0	0	0	10	n	N		0	0	0		
7	53	M	RLL	1/22/2014	0	0	0	0	0	0	0	10	6	N		1	1	1		
8	26	M	LLL	3/20/2014	0	0	0	0	0	0	0	6	9	N		1	1	0		
9	35	M	RLL	2/15/2014	18	0	0	0	0	0	0	18	23	N		1	1	0		
10	32	M	LLL	3/24/2014	0	0	0	0	0	0	0	14	2	N		1	1	1		
11	26	F	LLL	4/6/2014	2	0	0	0	0	0	0	14	3	N		0	0	0		
13	29	M	LLL	4/10/2014	17	0	0	0	0	0	0	17	64	N		1	1	1		
14	35	M	RLL	4/15/2014	0	0	0	0	0	0	0	6	78	N		0	0	0		
15	45	M	LLL	4/15/2014	18	0	0	0	0	0	0	28	32	N		1	1	0		
16	39	M	LUL	4/19/2014	0	1	1	1	0	0	0	6	6	N		1	1	1		
17	52	M	LLL	4/20/2014	0	0	0	0	0	0	0	10	10	N		1	1	0		
20	55	M	LLL	4/28/2014	2	1	1	0	0	0	0	10	7	N		1	1	1		
21	35	F	RLL	5/5/2014	1	1	1	1	0	0	0	11	2	N		1	1	1		
22	25	F	RLL	5/9/2014	10	1	1	1	0	0	0	18	20	N		1	1	0		
23	59	F	RLL	5/10/2014	0	0	0	0	0	0	0	10	n	N		1	1	0		
24	47	M	RLL	5/16/2014	24	1	1	1	0	0	0	34	44	N		1	1	0		
25	65	F	LUL	4/27/2014	0	1	1	0	0	0	0	10	36	N		1	1	1		
26	50	M	RLL	5/23/2014	2	1	1	0	0	0	0	10	7	N		0	0	0		
28	16	F	RUL	5/24/2014	16	1	1	0	0	0	0	32	29	N		1	1	0		
29	21	M	O	5/24/2014	20	0	0	0	0	0	0	44	36	N		0	0	0		
30	25	F	RLL	5/27/2014	10	0	0	0	0	0	0	37	2	N		1	1	1		
31	40	F	T	6/1/2014	5	0	0	0	0	0	0	5	1	N		1	1	0		
32	45	M	RLL	6/4/2014	10	1	1	0	0	0	0	20	4	N		1	1	1		
33	25	M	LLL	6/8/2014	2	0	0	0	0	0	0	20	n	N		1	1	0		
34	35	M	LLL	6/10/2014	8	0	0	0	0	0	0	17	3	N		1	1	0		
35	30	M	LLL	6/17/2014	8	1	1	0	0	0	0	10	3	N		1	0	0		
36	43	M	LLL	6/19/2014	0	0	0	0	0	0	0	20	1	N		1	1	0		
37	26	M	T	6/21/2014	9	0	0	0	0	0	0	17	38	N		1	1	0		
38	62	M	RLL	6/27/2014	8	0	0	0	0	0	0	24	12	N		1	1	1		
39	24	M	LUL	7/5/2014	N	0	0	0	0	0	0	N	7	Y	Cobra	0	0	0		
40	23	M	LLL	7/5/2014	10	0	0	0	0	0	0	18	5	N		1	1	1		
41	28	F	RLL	7/12/2014	N	0	0	0	0	0	0	16	6	Y	Russels	1	1	1		
42	25	F	LUL	7/13/2014	10	0	0	0	0	0	0	17	7	N		0	0	0		
43	23	F	LLL	7/13/2014	N	0	0	0	0	0	0	10	58	N		0	0	0		
44	44	M	LLL	7/19/2014	0	1	1	1	0	0	0	14	2	N		0	0	0		
45	45	F	RUL	7/16/2014	6	1	1	1	1	1	1	13	6	N		0	0	0		
46	58	F	RUL	7/7/2014	0	0	0	0	0	0	0	0	1	Y	Indian V	0	0	0		
47	21	F	LUL	7/15/2014	13	1	1	0	0	0	1	15	32	N		0	0	0		
48	24	M	U	7/19/2014	0	0	0	0	0	0	0	10	N	N		1	1	1		
49	21	M	LLL	8/4/2014	6	0	0	0	0	0	0	16	26	N		1	1	1		
50	68	M	LLL	8/4/2014	3	1	1	1	0	0	0	13	17	N		0	0	0		
51	68	F	RLL	8/1/2014	0	1	1	1	0	0	0	10	6	N		1	1	1		
52	19	F	RUL	8/8/2014	4	0	0	0	0	0	0	15	4	N		1	1	1		
53	33	M	RLL	7/26/2014	30	0	0	0	0	0	0	30	127	N		1	1	1		
54	50	M	RLL	8/3/2014	3	1	1	0	0	0	0	9	185	N		0	0	0		
55	48	M	RLL	8/11/2014	5	1	1	0	0	0	0	14	2	N		0	0	0		
56	19	M	LLL	8/17/2014	0	1	0	1	0	0	0	20	2	N		0	0	0		
57	45	M	RLL	8/21/2014	5	1	1	1	0	0	0	17	6	N		1	1	1		
58	28	M	RLL	8/22/2014	6	1	1	1	0	0	0	26	12	N		1	1	1		

Limb w	Duratio	Duratio	Duratio	Duratio	Haemo	WBCT	Pt Valu	Aptt Va	Pt -1/2	Aptt - 1	CPK	LDH	Sympto	Severity	Duratio	Thromb	DIC	Product	Abdom	Renal fa	No of D
	0	0			0	<10 mir	10.4	29.2	0.94	0	115	0	0	0		0	0	0	0	0	0
	0	0			0	<10 mir	10.6	28.5	0.96	0	100	0	0	0		0	0	0	0	0	0
	1	0			0	<20 m	13.5	26.5	11.9	0	0	0	0	0		0	0	0	0	0	0
	0	0			1	> 20 m	> 3 min	> 3 min	18.6	92.5	434	762.8	1	1		1	1	1	0	1	1
	0	0			1	<10 mir	13.4	24.7	11.9	0	421	5081	1	1		1	1	1	0	1	3
	0	0			1	> 20 m	47.2	17	14	0	2169	0	1	1		0	1	1	0	1	3
	1	1			1	<10 mir	1.7	33.3	12.5	0	960	0	1	1		1	1	0	0	1	0
	1	0			1	<10 mir	12.7	27.5	11.5	0	2128	0	1	1		1	1	0	0	1	4
	1	0			1	> 20 m	15.1	28	11.9	0	6449	875	1	1		0	0	0	0	1	0
	1	1			1	> 20 m	> 2 min	> 3 min	15.5	20.3	396	0	1	1		1	1	1	0	1	0
	0	0			1	< 20 m	12.5	27		0	146	0	1	1		0	0	0	0	0	0
	5	5			1	< 20 m	13.5	40.7	30	0	1436	546	1	1		1	1	0	0	0	0
	0	0			1	< 20 m	15.6	36.4	11.8	0	7761	1394	1	1		1	0	1	0	1	5
	4	0			1	> 20 m	13.8	31.3	11.5	0	853	1370	1	1		1	0	0	0	1	4
	4	4			1	< 20 m	11.5	32	0	0	960	0	1	1		0	0	0	0	0	0
	3	0			1	< 20 m	67.8	35.2	14.5	0	317	0	0	0		1	0	0	0	0	0
	4	4			1	< 20 m	13	68	12.2	0	257	0	1	1		0	0	0	0	0	0
	1	1			1	> 20 m	61.4	26.2	13.4	0	1520	1614	1	1		1	1	1	0	0	0
	1	0			1	> 20m	15.2	31.1	11	0	2662	961	1	1		0	0	0	0	1	0
	1	0			1	< 20 m	30.1	30.7	0	0	342	0	1	1		0	1	1	0	1	0
	3	0			1	> 20 m	18.9	37	12.2	0	6611	2619	1	1		1	1	1	1	1	1
	4	4			1	< 20 m	11.9	25	10.8	0	0	0	0	0		0	0	0	0	0	0
	0	0			0	< 20 m	13.5	25.4	11.5	0	2189	0	0	0		0	0	0	0	0	0
	1	0			0	< 10 m	13.7	28.4	11.5	0	0	0	0	0		0	0	0	0	0	0
	0	0			1	> 20 m	27.3	41.1	12.2	30.3	7654	2166	1	1		1	1	1	1	1	1
	5	5			1	< 10 m	28	28.7	13.7	0	27121	0	1	1		1	1	1	1	0	0
	1	0			0	< 10 m	10.9	25.7	0.99	0	194	0	0	0		0	0	0	0	0	0
	5	5			1	>20 m	32.4	40.9	13.9	27	1225	4799	1	1		0	0	0	0	1	0
	1	0			1	> 20 m	> 2 min	> 3 min	14.3	36.4	2118	0	1	1		1	1	0	0	1	0
	3	0			1	< 10 m	19.8	111.4	11.8	44.2	11380	0	1	1		1	1	0	0	1	0
	0	0			1	> 20 m	> 2 min	> 3 min	88.2	0	936	0	1	1		0	1	0	0	0	0
	3	0			1	> 20 m	29.3	92	14	71.6	138	0	1	1		1	1	1	1	0	0
	3	0			0	< 20m	13.1	25	11.3	0	129	0	0	0		0	0	0	1	0	0
	1	1			1	< 12 m	51	38.3	13.4	25	805	0	1	1		1	1	0	1	0	0
	0	0			0	< 10 m	12.5	30.7	0	0	315	0	0	0		0	0	0	1	0	0
	5	5			1	< 10 m	62	26	0	0	920	0	1	1		1	1	0	0	0	0
	4	4			1	< 20 m	24.5	26	13.2	0	566	0	0	0		0	0	0	0	0	0
	0	0			1	< 20 m	21.4	25.4	11.4	0	455	0	0	0		1	0	0	0	0	0
	0	0			1	> 20 m	16.1	36.2	11.6	0	286	0	0	0		1	0	0	1	0	0
	0	0			1	< 20 m	31	32.1	12	0	3368	0	1	1		1	1	1	0	0	0
	0	0			1	< 20 m	25.7	29.2	12.1	0	0	0	0	0		1	1	0	1	0	0
	0	0			0	< 20 m	10.1	25	0	0	0	0	0	0		0	0	0	0	0	0
	0	0			1	> 20 m	19	37.7	11.9	27.9	951	0	1	1		1	1	0	0	0	0
	3	3			0	< 20 m	11.4	26.2	0	0	319	0	0	0		0	0	0	1	0	0
	3	3			1	> 20 m	11.9	24.8	0	0	4682	3520	1	1		1	1	0	0	0	0
	0	0			1	> 20 m	>2 mins	> 3 min	13.1	24	752	709	0	0		1	1	1	0	0	0
	2	2			1	< 20 m	> 2mins	> 3 min	12.4	18	226	5560	1	0		1	0	0	0	1	2
	4	4			0	< 10 m	13.2	27.6	10.9	0	258	0	0	0		0	0	0	1	0	0
	8	6			1	< 20 m	11.3	26.9	0	0	2041	2014	1	1		1	1	1	0	1	1
	0	0			1	> 20 m	19.1	29	0	0	135	0	1	1		1	0	0	0	0	0
	0	0			1	> 20 m	> 2mins	110	33	0	0	0	1	0		0	1	0	0	0	0
	0	0			1	< 20 m	> 2mins	33.4	11.2	0	489	0	0	0		1	1	0	0	1	0
	2	2			1	> 20 m	> 2mins	> 3 min	11.8	31.4	0	0	1	0		0	1	0	0	0	0
	10	10			1	> 20 m	33.8	56.8	12.4	31	9875	861	1	1		1	1	1	0	1	1

HUS	Rhabdo	Local Sv	Cellulitis	Necroti	Duratio	Outcom	Syndro	Complic	Unusual syndromes				LAB NO	Name	NO OF A	PREVIO	SYNDRO	D1 AVG	D2 AVG	D3 AVG	D4 AVG	D5 AVG	D6 AVG
0	0	1	0	0	0	1	2																
0	0	0	0	0	0	1	1																
0	0	1	0	0	0	1	2																
0	0	1	1	0	2	1	9																
1	1	1	1	0	3	1	11																
0	1	1	1	0	0	1	8																
0	1	1	1	0	0	1	7																
0	1	1	1	0	3	1	9																
0	1	1	1	0	0	1	7																
0	1	1	1	0	6	1	9																
0	0	1	1	1	0	1	7																
0	1	1	0	0	3	1	9																
0	1	1	0	0	5	1	9																
0	1	1	1	0	0	1	9																
0	0	0	0	0	5	1	6																
0	0	1	1	0	0	1	7																
0	0	1	1	0	0	1	7																
0	1	1	1	1	2	1	7																
0	1	1	1	0	0	1	9																
0	1	1	1	0	0	1	9																
0	1	1	1	0	4	0	9																
0	0	1	1	0	4	1	7																
0	1	1	1	0	0	1	3																
0	0	1	1	0	0	1	6																
1	1	1	1	0	10	0	11																
0	1	1	1	0	1	1	7																
0	0	1	1	0	0	1	6																
0	1	1	1	0	0	1	9																
0	1	1	1	0	0	1	9																
0	1	0	0	0	2	1	9																
0	1	1	1	0	0	1	7																
0	0	1	1	0	0	1	7																
0	0	0	0	0	5	1	5																
0	1	1	1	0	0	1	7																
0	0	1	1	0	0	1	13																
0	1	1	1	0	3	1	7																
0	1	1	1	0	0	1	7																
0	0	1	1	0	0	1	3																
0	0	1	1	0	0	1	3																
0	1	1	1	0	0	1	3																
0	0	1	1	0	0	1	3																
0	0	0	0	0	0	1	1																
0	1	1	1	0	0	1	13	myocarditis															
0	0	0	0	0	3	1	5																
0	0	1	1	0	0	1	7																
0	1	1	1	0	2	1	7																
0	1	1	1	0	2	0	9																
0	0	1	1	0	0	1	6																
1	1	1	1	0	9	2	13	HT,NT,RF,CVA,LOCAL SWELLING															
0	0	1	1	0	0	1	3																
0	0	1	1	0	0	1	3																
0	1	1	1	0	0	1	11	myocarditis															
0	0	1	1	0	0	1	7																
0	1	1	1	1	11	0	7	Septic shock															

59	35	M	LUL	8/26/2014	6	1	1	1	1	0	0	16	40	Y		1	1	0		
60	40	M	LLL	9/3/2014	5	1	1	1	0	0	0	15	15	N		0	0	0		
61	38	M	RLL	9/3/2014	2	1	1	1	0	0	0	12	5	N		1	1	0		
62	44	M	RLL	9/3/2014	0	0	0	0	0	0	0	10	n	N		1	1	0		
63	36	M	LLL	9/7/2014	0	0	0	0	0	0	0	0	2	N		0	0	0		
64	45	F	RUL	9/6/2014	5	0	0	0	0	0	0	20	3	N		1	1	0		
65	54	F	LUL	9/13/2014	0	0	0	0	0	0	0	8	1	Y		0	0	0		
66	28	F	LLL	9/18/2014	2	0	0	0	0	0	0	20	4	Y	Commo	1	1	1	1	1
67	22	M	RLL	9/18/2014	21	0	0	0	0	0	0	23	41	N		1	1	0	1	1
68	65	F	RLL	9/20/2014	20	0	0	0	0	0	0	20	28	N		0	0	0	0	0
69	20	F	LUL	9/24/2014	2	0	0	0	0	0	0	22	12	Y	Russels	1	1	0	0	0
70	24	M	RUL	9/25/2014	8	0	0	0	0	0	0	8	6	Y	Cobra	1	0	0	1	1
71	39	M	LLL	9/27/2014	16	1	1	1	1	1	1	16	44	N		0	0	0	0	0
72	40	M	LLL	9/27/2014	20	0	0	0	0	0	0	21	50	N		0	0	0	0	0
73	40	M	RLL	9/29/2014	6	1	1	1	1	1	1	16	36	N		0	0	0	0	0
74	51	F	LLL	10/8/2014	2	1	1	1	0	0	0	12	5	N		0	0	0	0	0
75	60	M	RLL	10/13/2014	8	1	1	1	0	0	0	10	9	N		1	1	0	1	1
76	43	M	RUL	10/15/2014	0	0	0	0	0	0	0	10	2	N		0	0	0	0	0
77	54	M	RUL	10/16/2014	8	0	0	0	0	0	0	16	3	N		0	0	0	0	0
78	47	M	RUL	10/16/2014	0	0	0	0	0	0	0	10	2	N		0	0	0	0	0
79	45	M	LLL	10/18/2014	0	0	0	0	0	0	0	14	7	N		1	1	1	0	0
80	56	M	SCROTUM	10/24/2014	0	0	0	0	0	0	0	10	12	N		1	1	1	1	1
81	47	F	RLL	10/24/2014	3	1	1	1	0	0	1	13	5	N		1	1	1	0	0
82	60	F	LUL	11/2/2014	4	1	1	0	0	0	0	18	8	N		0	0	0	0	0
83	25	M	RLL	11/11/2014	8	0	0	0	0	0	0	20	2	N		1	1	1	0	0
84	24	F	LLL	11/16/2014	6	0	0	0	0	0	0	16	15	N		1	1	1	0	0
85	30	M	LLL	12/6/2014	18	0	0	0	0	0	0	18	4	N		1	1	0	0	0
86	55	M	RLL	12/9/2014	20	0	0	0	0	0	0	20	6	N		1	1	1	1	1
87	60	F	RUL	12/11/2014	20	0	0	0	0	0	0	30	1	N		1	1	1	1	1
88	35	F	LLL	12/19/2014	5	1	1	1	0	1	0	15	2	N		1	1	1	0	1
89	54	M	LLL+RLL	12/19/2014	10	0	0	0	0	0	0	24	2	N		1	1	1	0	1
90	25	M	RLL	1/4/2015	10	1	1	0	0	1	0	22	7	N		1	1	1	0	0
91	35	F	RLL	1/21/2015	2	1	1	1	1	0	0	12	6	N		1	1	1	0	0
92	62	M	RUL	1/20/2015	10	0	0	0	0	0	0	14	5	N		0	0	0	0	0
93	48	M	LLL	2/1/2015	8	0	0	0	0	0	0	18	4	N		1	1	1	1	1
94	37	M	RLL	2/5/2015	0	1	1	1	1	1	1	10	3	N		0	0	0	0	0
95	19	M	RUL	2/3/2015	10	1	1	1	0	0	0	20	4	N		0	0	0	0	0
96	51	M	RLL	2/13/2015	0	1	1	1	0	0	0	10	4	N		1	1	1	0	0
97	50	F	RLL	2/13/2015	4	1	1	1	1	0	1	14	5	N		0	0	0	0	0
98	24	M	LLL	2/14/2015	10	0	0	0	0	0	0	20	3	N		1	1	1	1	1
99	16	F	RLL	2/18/2015	10	0	0	0	0	0	0	20	2	N		1	1	1	0	0
100	60	M	RUL	2/20/2015	NA	0	0	0	0	0	0	14	54	N		0	0	0	0	0
101	30	F	LLL	2/27/2015	20	0	0	0	0	0	0	30	1	N		1	1	1	1	1
102	32	F	RLL	3/3/2015	25	1	1	1	0	0	0	29	4	N		1	1	1	1	1
103	24	M	LLL	3/13/2015	8	0	0	0	0	0	0	18	2	N		1	1	1	0	0
104	27	M	LLL	3/15/2015	12	0	0	0	0	0	0	20	1	N		1	1	1	1	1
105	30	F	RLL	2/21/2015	4	0	0	0	0	0	0	20	3	Y	Russels	0	0	0	0	0
106	31	M	LLL	3/21/2015	NA	0	0	0	0	0	0	13	6	N		1	1	1	1	0
107	28	M	LLL	4/5/2015	26	1	1	1	1	1	1	36	1	N		1	1	1	0	0
108	43	M	LUL	4/7/2015	1	0	0	0	0	0	0	11	8	N		1	1	1	0	0
109	34	M	FACE	4/7/2015	11	0	0	0	0	0	0	17	3	N		1	1	1	1	1
110	36	M	LLL	4/8/2015	25	1	1	1	0	1	0	35	4	N		1	1	1	1	1
111	26	M	RUL	4/17/2015	1	0	0	0	0	0	0	13	5	N		1	1	1	0	1
112	18	M	RUL	4/20/2015	0	0	0	0	0	0	0	10	14	N		1	1	1	0	0
113	35	M	LLL	4/22/2015	6	1	1	0	0	0	0	22	1	N		1	1	1	0	0
114	51	F	Trunk-glute	4/24/2015	0	0	0	0	0	0	0	10	6	N		1	1	1	0	0
115	55	F	RUL	4/26/2015	10	0	0	0	0	0	0	18	3	Y	Commo	1	1	1	0	0

	2	0			1	< 15 m	14.9	13.8	11.7	0	0	0	0	0		1	0	0	0	1	0
	0	0			1	> 20 m	> 2mins	> 3mins	13.9	25	0	0	1	1		0	1	0	0	0	0
	3	0			0	< 10 m	11.6	26	0	0	0	0	0	0		1	0	0	0	0	0
	2	0			0	< 10 m	10.3	28.4	0	0	0	0	0	0		0	0	0	0	0	0
	0	0			0	< 10 m	10.3	25.7	0	0	83	432	0	0		0	0	0	0	0	0
	3	0			1	> 20 m	> 2 mins	> 3 mins	12	25	5341	0	1	1		1	1	1	0	0	0
	0	0			1	> 20 m	34.7	58.9	18	0	1713	7620	1	1		1	1	1	0	1	3
1	5	5	5	3	0	<10 min	12.6	34	77.9	0	96.2	0	0	0	0	0	0	0	0	0	0
0	4	0	6	0	1	> 20 m	22.4	43.7	12.4	29.7	0	>2000	1	1	10	1	1	1	0	1	4
0	0	0	0	0	1	< 15 m	16.6	37.5	13	33.7	3375	2767	1	0	4	1	1	1	0	1	4
0	1	0	0	0	1	> 20 m	30.3	34.8	11.8	0	396	0	0	0	1	0	1	0	0	0	0
0	0	0	2	0	0	< 5 m	12.8	23.1	10.8	0	322	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	< 10 m	17.2	31.7	11.5	0	460	614.9	0	0	4	1	1	0	0	0	0
0	0	0	0	0	1	> 20 m	12.4	24.4	1.12	0	148	618	1	1	5	1	1	0	0	1	0
0	0	0	0	0	1	> 20 m	23.2	29.6	12.3	0	2634	0	1	1	5	1	1	1	0	1	1
0	0	0	0	0	1	> 20 m	clot	clot	clot	clot	88	0	0	0	1	0	1	0	1	0	0
1	2	0	3	2	0	< 10 m	11.8	22.8	0	0	80	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	1	> 20 m	> 2 min	> 3 mins	12.5	25	296	0	1	1	1	0	1	0	1	0	0
0	0	0	0	0	1	> 20 m	18.7	35.5	11.2	0	0	0	1	0	1	1	1	0	0	0	0
0	0	0	0	0	1	> 20 m	> 2 mins	43.4	13.6	25	720	0	0	0	2	1	1	0	0	0	0
0	3	3	0	0	1	> 20 m	> 2 mins	31	13.4	0	0	0	0	0	1	1	1	0	0	0	0
0	2	3	2	0	0	< 10 m	10.1	1.2	0	0	0	0	0	0	0	0	0	0	0	0	0
0	2	2	0	0	0	< 15 m	14.1	27.2	11.5	0	299	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	< 20 m	16.6	24	11.5	0	0	0	1	1	1	0	1	1	0	0	0
0	2	2	0	0	1	> 20 m	15.7	243	12.3	0	0	0	0	0	1	0	1	0	0	0	0
0	2	2	0	0	1	< 20 m	17.7	25	0	0	4916	2398	1	1	1	0	0	0	0	0	0
0	2	0	0	0	1	< 10 m	15.5	27.5	11.6	0	9333	2239	1	1	1	1	1	0	0	1	0
1	4	5	2	2	1	> 20m	23.9	34.8	12.8	0	23200	5200	1	1	2	1	1	1	0	1	10
0	3	4	4	2	0	< 10 m	11.6	24.3	0	0	370	0	0	0	0	0	0	0	0	0	0
1	2	2	0	2	0	< 10 m	10	24	0	0	66	0	0	0	0	0	0	0	0	0	0
0	5	6	0	0	1	< 20 m	31.6	44.4	13.5	33	1908	1984	1	1	3	1	1	1	0	0	0
0	3	3	0	0	0	< 10 m	11.6	30.2	0	0	0	0	0	0	0	0	0	0	1	0	0
0	2	3	0	0	1	> 20 m	12.4	32.2	11.7	0	256	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	1	< 20 m	11.9	24.3	0	0	70	726	0	0	6	1	0	0	0	1	0
0	4	5	4	0	1	> 20 m	54.2	34.2	15.4	0	1261	2128	1	1	5	1	1	1	0	1	4
0	0	0	0	0	1	> 20 m	10.7	28.2	11.8	30.2	139	0	0	0	3	0	1	0	0	0	0
0	0	0	0	0	1	< 20 m	12.4	25.6	0	0	320	2783	1	1	1	1	1	0	1	1	5
0	4	5	0	0	1	< 20 m	14	36.8	11	0	61	0	0	0	2	0	0	0	0	1	0
0	0	0	0	0	1	> 20 m	22.7	64	12.4	33.2	120	504	1	1	5	1	1	1	0	1	4
1	3	4	2	2	1	> 20 m	23.8	52.3	11.9	35.7	42088	0	0	0	5	1	1	1	1	1	5
0	4	5	0	0	1	> 20 m	22.3	28.7	11.9	0	966	0	0	0	3	0	0	0	0	0	0
0	0	0	0	0	1	> 20 m	clot	clot	clot	clot	417	1025	1	1	3	1	1	1	0	1	0
1	2	2	2	2	1	> 20 m	20	27.4	12.1	0	1081	2741	1	1	3	1	1	1	0	1	3
0	5	5	2	0	1	> 20 m	13.5	31.1	10.7	0	3720	1155	1	1	5	1	1	1	0	1	1
0	3	3	0	0	1	< 10 m	15.4	24.2	11.9	0	415	0	1	1	1	0	0	0	1	0	0
0	4	5	1	0	1	> 20 m	18	30.4	12.4	0	3883	0	0	0	2	1	1	0	0	0	0
0	0	0	0	0	1	> 20 m	> 2 mins	> 3 mins	0	36	238	0	1	0	1	0	1	0	0	0	0
0	4	5	2	0	1	> 20 m	20.7	49.3	12.7	34.4	1523	2146	1	1	5	1	1	1	1	1	5
0	3	4	0	0	1	> 20 m	23	160.1	11.7	83.7	8032	0	1	1	4	1	1	1	1	1	3
0	3	4	0	0	1	> 20 m	22.3	25.6	13	0	0	505	1	1	2	1	1	0	0	0	0
0	5	5	3	2	1	< 20 m	14.1	51.3	11	39.6	1546	1011	0	0	5	1	1	0	1	1	0
1	4	5	4	3	1	> 20 m	15.6	27.9	11.4	0	3296	1502	1	1	5	1	1	1	0	1	4
0	4	5	0	0	0	< 20 m	11.7	35.7	0	0	3214	0	0	0	0	0	0	0	0	0	0
0	4	4	0	0	0	< 20 m	12.2	33	0	0	547	0	0	0	0	0	0	0		0	0
0	4	5	0	0	1	> 20 m	68	32	13	0	619	1609	1	1	1	0	1	0		1	4
0	3	3	0	0	1	> 20 m	35	31.1	12	0	219	0	1	1	1	1	1	0		0	0
0	3	4	0	0	0	< 20 m	10.8	31.5	0	0	300	310	0	0	0	0	0	0	1	0	0

0	0	1	1	0	0	2	9															
0	0	1	1	0	0	1	3															
0	0	1	1	0	0	1	6															
0	0	1	1	0	0	1	6															
0	0	0	0	0	0	1	1															
0	1	0	0	0	20	1	8															
0	1	1	1	0	0	1	11															
0	0	0	0	0	9	1	5															
0	1	1	1	0	5	1	9					2	PASUPA	8	13	HT+RF+	0.752	0.517	0.473	0.433	0.43	
0	1	1	1	0	0	1	11					3	SUBATH	N	20	HT+LS	0.426	0.407	0.626	0.546	0.464	
0	0	1	1	0	0	1	7					4	INDUM	D1-10, I	N		0.294	0.318	0.255			
0	0	1	1	0	0	1	6					5	KRISHN	N	8	LS	0.278	0.336	0.337			
0	0	1	1	0	0	1	3	Shock				6	MURUG	N	8	HT+LS	0.473	0.385	0.391	0.307	0.575	
0	1	1	1	0	0	1	11					7	MANI	6	20		0.283	0.281	0.255	0.239	0.287	
0	1	1	1	0	21	1	11					8	MOHAN	10	6	HT+RF+	0.528	0.532	0.482	0.458	0.406	
0	0	1	1	0	0	1	3					9	CHAND	D1-10, I	2	HT+NT+	0.253	0.258	0.275			
0	0	0	0	0	0	1	5					10	BALU	10	N	NT	0.287	0.287	0.254	0.292		
0	0	1	1	0	0	1	3					11	MOHAN J									
0	0	1	1	0	0	1	3					12	KUMAR	2	8	HT+NT+	0.568	0.526				
0	0	1	1	0	0	1	3					13	RAVIKUMAR				0.388	0.226	0.246	0.292	0.281	
0	0	1	1	0	0	1	7					14	CHINNA	D1-8, D	N	HT+NT+	0.468	0.452	0.457	0.445	0.394	
0	0	1	1	0	0	1	6					15	PARASI	10	N	NT	0.312	0.247	0.244	0.216	0.211	
0	1	1	1	0	0	1	6					16	PUSHPA	24.10.1	N	NT+LS	0.672	0.545	0.5	0.318	0.429	
0	0	1	1	1	0	1	3					17	KUPPAMAL				0.493	0.479	0.28	0.643	0.554	
0	0	1	1	0	0	1	7					18	SRINIVASAN				0.602	0.428	0.429			
0	1	1	1	0	0	1	7					19	SASIKAL	10	6	HT+NT+	0.296	0.296	0.273	0.59	0.624	
1	1	1	1	0	0	1	9					20	VENKAT	N	17	HT+NT+	0.253	0.29				
1	1	1	1	1	9	1	9					21	MANI	6	20		0.611	0.622	0.468			
0	0	1	1	0	2	1	6					22	PAVADA	10	20	NT+LS	0.243	0.295	0.305			
0	0	1	1	0	0	1	6					23	VARALAKSHMI				0.519	0.478	0.367	0.373		
0	1	1	1	0	0	1	7					24	GOVIND	10	10	HT+NT+	0.242	0.269	0.232	0.32	0.511	
0	0	0	0	0	0	1	5					25	VIGNES	D1-6, D	8	HT+NT+	0.279	0.302				
0	0	1	1	0	0	1	7					26	SUJATH	10	2	HT+NT+	0.564	0.552	0.471	0.487		
0	0	1	1	0	0	1	11					27	SUNDRA	4	10	HT+LS	0.439	0.429	0.431	0.416	0.582	
1	1	1	1	0	7	1	9	VAP	ET BLEED,SECONDARY H			28	ANJANA	10	8	HT+NT+	0.706	0.523	0.535	0.501	0.475	0.445
0	0	1	1	0	0	1	3	ASV ANAPHYLACTIC SHOCK				29	SRINIVASAN				0.328	0.666	0.611	0.566		
1	1	1	1	0	6	1	11	Enterococcal sepsis				30	MUNISAMY				0.484	0.484	0.601	0.481	0.36	
0	1	1	1	0	0	1	9					31	SUBRAM	10	N	NT+LS	0.848	0.828	0.81	0.756		
1	1	1	1	0	0	1	11	Shock				32	SARASV	10	4	HT+RF+	0.709	0.662	0.611	0.587	0.88	
1	1	1	1	0	6	1	9	Pancrea	Shock liver.NFGNB sepsi			33	RANJIT				0.848	0.813	0.901	0.742	0.667	
0	1	1	1	0	0	1	7					34	DIVYA	10	10	HT+NT+	0.749	0.609	0.886	0.873	0.792	
1	1	1	1	0	0	1	11	Right lo	H1N1 superinfection			35	SRIRAM	D1-10, I	N	HT+RF+	0.787	0.81	0.771	0.691	0.659	
1	1	1	1	0	2	0	9	Cardiogenicseptic shock, myocar				36	AMBIKA				0.845	0.757	0.732	0.683		
1	1	1	1	0	9	1	9					37	SELVI	4	18	NT+SEP	0.797	0.76	0.76	0.637	0.666	
0	1	1	0	0	0	1	7					38	TAMILS	10	8	HT+NT+	0.883	0.782	0.658	0.483		
0	1	1	1	0	0	1	7					39	SRIDHA	8	12	HT+NT+	0.771	0.665	0.582	0.855	0.835	
0	0	1	0	0	0	1	3	RTA				40	PUSHPA	6	4	HT+LS	0.804	0.757	0.76	0.744	0.71	
1	1	1	1	1	5	1	9	Sheehans syndrome				41	KUMAR				0.523	0.848	0.847	0.738	0.739	
0	1	1	1	0	5	1	9	Uraemic pleural effusion				42	SOLLAR	10	26		0.963	0.797	0.662	0.644	0.905	0.888
0	0	1	1	0	0	1	7					43	PALANI				0.403	0.331	0.352	0.331	0.421	
0	1	1	1	0	4	1	9	Cardiogenicseptic shock, myocar				44	GURUV	D1-6, D	1	HT+NT+	0.379	0.431	0.244	0.351	0.269	
1	1	1	1	0	5	1	9	Sheeha	acute angle closure glau			45	PONNUSAMY				0.271	0.272	0.289	0.299	0.283	
0	1	0	0	0	2	1	5	VAP				46	DEVARAJALU				0.349	0.324	0.315	0.305		
0	0	1	1	1	0	1	6					47	SARATH KUMAR				0.28	0.346	0.304	0.236	0.28	
1	1	1	1	0	2	1	9	ESBL E coli UTI				48	SANJU REDDY				0.311	0.264	0.24	0.275	0.289	
0	1	1	1	0	0	3	7	Cardiogenic shock with myocard				49	MAHESHWARI				0.37					
0	0	0	0	0	0	1	5					50	VALARMATHI				0.329	0.385	0.282	0.299		

116	18	M	RLL	5/5/2015	0	1	1	1	0	0	1	10	2	N		1	1	1	1	1
117	28	M	RUL	5/5/2015	4	1	1	1	1	1	0	26	2	N		1	1	1	1	0
118	31	M	LLL	5/10/2015	0	0	0	0	0	0	0	0	NA	N		0	0	0	0	0
119	32	F	LLL	5/14/2015	4	0	0	0	0	0	0	14	6	N		1	1	1	1	1
120	15	M	RUL	5/13/2015	23	0	0	0	0	0	0	23	4	N		1	1	1	1	1
121	54	M	RUL	5/16/2015	NA	0	0	0	0	0	0	12	4	N		0	0	0	0	0
122	29	M	LLL	5/20/2015	0	1	1	1	1	1	1	10	8	N		1	1	1	0	0
123	38	M	RUL	5/21/2015	4	1	1	1	1	1	0	14	1	N		1	1	1	0	0
124	30	F	RUL	5/21/2015	7	0	0	0	0	0	0	13	1	N		1	1	1	1	1
125	47	M	LLL	5/29/2015	0	0	0	0	0	0	0	16	3	N		1	1	1	1	1
126	40	M	RLL	5/30/2015	5	1	1	1	1	1	1	15	2	N		1	1	1	1	1
127	25	M	RLL	6/6/2015	10	0	0	0	0	0	0	30	1	N		1	1	1	1	1
128	30	M	RUL	6/4/2015	24	0	0	0	0	0	0	24	2	N		0	0	0	0	0
129	35	M	RLL	6/8/2015	5	0	0	0	0	0	0	15	2	N		1	1	1	0	0
130	56	M	RLL	6/10/2015	0	0	0	0	0	0	0	10	2	N		1	1	1	0	0
131	39	M	LLL	6/24/2015	6	0	0	0	0	0	0	16	6	N		0	0	0	0	0
132	36	F	Unknown	7/1/2015	NA	1	1	1	1	1	1	10	2	N		1	1	1	1	1
133	31	M	LUL	7/2/2015	4	1	0	0	0	1	0	14	2	N		1	1	1	0	1
134	40	M	LLL	7/7/2015	10	0	0	0	0	0	0	20	3	N		1	1	1	1	1
135	60	M	RUL	7/14/2015	10	1	1	1	0	1	0	23	2	N		1	1	1	0	0
136	54	F	RLL	7/18/2015	0	1	1	1	1	1	1	20	1	N		0	0	0	0	0
137	55	F	LLL	7/22/2015	0	0	0	0	0	0	0	0	NA	Y		0	0	0	0	0
138	48	F	RLL	7/21/2015	18	1	1	1	1	1	1	28	6	N		1	1	1	1	1
139	46	M	RLL	7/24/2015	3	1	1	1	1	1	1	23	2	N		1	1	0	1	0
140	32	M	RLL	7/25/2015	6	0	0	0	0	0	0	16	3	N		1	1	1	1	0
141	45	M	LLL	7/31/2015	40	0	0	0	0	0	0	40	4	N		1	1	1	0	0
142	63	F	LLL	8/2/2015	16	0	0	0	0	0	0	16	2	Y		1	1	1	1	1
143	35	F	LUL	8/6/2015	0	0	0	0	0	0	0	2	30	N		0	0	0	0	0
144	28	F	LLL	8/6/2015	0	0	0	0	0	0	0	10	2	N		0	0	0	0	0
145	18	M	RLL	8/5/2015	NA	0	0	0	0	0	0	10	NA	N		1	1	1	1	1
146	45	F	RLL	8/8/2015	4	1	1	1	1	1	1	14	1	N		0	0	0	0	0
147	45	F	LUL	8/9/2015	0	0	0	0	0	0	0	10	2	Y	Cobra(li	1	1	1	1	1
148	40	M	LLL	8/7/2015	20	0	0	0	0	0	0	30	2	N		1	1	1	0	0
149	52	F	RLL	8/8/2015	0	0	0	0	0	0	0	10	3	Y	Russels	0	0	0	0	0
150	60	M	LLL	8/12/2015	23	0	0	0	0	0	0	23	3	N		1	1	1	1	0
151	40	F	LUL	8/14/2015	12	0	0	0	0	0	0	22	3	N		1	1	1	1	0
152	36	F	LUL	8/15/2015	3	0	0	0	0	0	0	13	4	N		1	1	1	0	0
153	20	M	RLL	8/20/2015	6	0	0	0	0	0	0	22	2	Y	Saw sca	0	0	0	0	0
154	36	M	LLL	8/23/2015	1	1	1	1	1	1	0	9	4	N		1	1	1	1	1
155	54	M	LUL	8/27/2015	10	0	0	0	0	0	0	10	6	N		0	0	0	0	0
156	65	F	RLL	9/2/2015	20	0	0	0	0	0	0	30	4	N		1	1	0	0	0
157	47	M	LLL	9/19/2015	NA	0	0	0	0	0	0	10	NA	N		1	1	1	1	1
158	20	M	RLL	9/17/2015	8	0	0	0	0	0	0	28	1	N		1	1	1	1	1
159	65	M	LLL	10/17/2015	0	0	0	0	0	0	0	10	4	N		1	1	1	1	0
160	50	M	RLL	10/4/2015	10	0	0	0	0	0	0	30	1	N		0	0	0	0	0
161	22	F	LLL	10/6/2015	10	0	0	0	0	0	0	20	3	N		1	1	1	0	0
162	30	M	LLL	10/19/2015	10	0	0	0	0	0	0	10	1	N		0	0	0	0	0
163	55	M	RLL	10/14/2015	18	0	0	0	0	0	0	0	2	0		0	0	0	0	0
164	46	M	RLL	10/21/2015	10	0	0	0	0	0	0	10	2	N		0	0	0	0	0
165	35	M	RLL	11/8/2015	25	0	0	0	0	0	0	25	6	N		1	1	1	0	1
166	37	F	RLL	11/25/2015	10	0	0	0	0	0	0	10	6	N		1	1	1	1	1
167	17	M	RUL	11/29/2015	8	0	0	0	0	0	0	18	3	N		0	0	0	0	0
168	46	M	RUL	12/30/2015	8	0	0	0	0	0	0	23	3	N		1	1	0	0	0
169	16	M	RLL	1/5/2016	8	0	0	0	0	0	0	10	2	N		1	1	1	0	0
170	54	F	RLL	1/11/2016	12	0	0	0	0	0	0	24	6	N		1	1	1	1	1
171	20	M	LLL	1/23/2016	10	0	0	0	0	0	0	18	3	N		1	1	1	1	1

0	3	3	3	0	1	> 20 m	17.8	39.3	11.5	0	0	0	1	1	4	1	1	1	0	1	8
0	4	5	2	0	1	> 20 m	87	29.3	0	0	4107	0	1	1	3	0	1	0	1	1	0
0	0	0	0	0	1	> 20 m	11.4	24	0	0	777	11600	1	1	5	1	1	1	1	1	13
1	4	5	3	3	0	< 10 m	10.7	31.8	0	0	94	0	0	0	0	0	0	0	0	0	0
0	4	5	4	0	1	< 20 m	Clot	clot	clot	clot	3073	0	1	1	1	1	1	1	0	0	0
0	0	0	0	0	1	< 20 m	18.2	26.5	13.1	0	3132	1161	0	0	1	0	0	0	0	0	0
0	3	4	0	0	1	< 15 m	35.9	45.3	12.2	37.3	0	0	0	0	3	1	0	0	0	0	0
0	2	2	0	0	1	> 20 m	16.1	62.5	11.4	55.4	0	0	1	1	1	0	1	0	0	0	0
0	4	5	2	0	0	> 20 m	13.4	26.2	11.5	0	696	0	0	0	0	0	0	0	0	0	0
0	3	4	4	0	1	> 20 m	>2 min	>3 min	14.1	70.6	375	0	1	1	2	1	1	0	0	1	0
0	3	4	2	0	1	> 20 m	24.5	33.3	12.3	0	610	0	1	1	4	1	1	1	0	1	0
0	4	5	1	0	1	> 20 m	39.2	36.1	12.9	0	0	0	1	1	1	1	1	1	0	0	0
0	0	0	0	0	1	> 20 m	15.2	52.7	11.4	47.5	50	412.9	1	1	3	1	1	0	0	1	0
0	5	5	0	0	1	> 20 m	25.2	34.5	0	0	19060	4340	1	1	4	1	1	0	1	1	7
0	2	2	0	0	0	< 20 m	10.2	36.7	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	> 20 m	35.2	61	11.6	37.5	96	827.4	1	1	2	1	1	1	0	0	0
0	4	5	4	4	0	< 20 m	9.9	31	0	0	0	0	0	0	0	0	0	0	0	0	0
1	3	3	0	3	0	< 20 m	10.9	28.7	0	0	0	0	0	0	0	0	0	0	0	0	0
0	3	3	2	0	1	> 20 m	59.2	30.6	13	0	0	0	0	0	1	0	0	0	0	0	0
0	4	5	0	0	1	< 20 m	11.9	36.8	11.2	0	252	0	1	0	1	0	0	0	0	0	0
0	0	0	0	0	1	> 20 m	>2 min	>3 min	12.5	25.5	143	0	0	0	1	0	1	0	0	0	0
0	0	0	0	0	0	< 10 m	9.9	31.1	0	0	260	0	0	0	0	0	0	0	0	0	0
0	3	4	1	0	1	> 20 m	13.7	34.3	11.1	0	1365	9460	1	1	6	1	1	1	1	1	4
0	2	0	2	0	1	> 20 m	>2min	>3min	0	0	321	756	1	1	1	1	1	0	1	0	0
0	1	1	1	0	0	< 20 m	9.9	35.3	0	0	0	0	0	0	0	0	0	0	0	0	0
0	2	2	0	0	1	< 20 m	10.9	36.5	0	0	914	0	1	1	1	1	1	0	0	0	0
1	3	4	1	1	0	< 20 m	11.3	25.1	0	0	5659	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	< 20 m	10.9	33	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	< 20 m	17.2	25.7	11.8	0	72	499	0	0	1	0	1	0	0	0	0
0	4	5	3	0	0	< 20 m	14.6	34.3	10.9	0	699	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	> 20 m	57.5	37.8	11.7	0	0	0	1	1	1	0	1	0	0	0	0
0	2	2	1	0	0	< 20 m	10.5	29.8	0	0	240	0	0	0	0	0	0	0	0	0	0
0	3	4	0	0	1	> 20 m	21.4	53.2	12.4	40.2	1	2340	1	1	5	1	1	0	0	1	6
0	0	0	0	0	1	> 20 m	22.1	44.4	12	28	69	0	0	0	1	0	1	0	0	1	0
0	4	5	1	0	1	< 20 m	11	32	0	0	656	1511	0	0	1	0	0	0	0	1	0
0	4	4	2	0	1	< 20 m	12	38	0	0	94	2225	0	0	1	1	1	0	0	1	0
0	2	3	0	0	1	> 20 m	>2 min	26.9	12.7	0	396	0	0	0	3	0	1	0	0	1	0
0	0	0	0	0	1	> 20 m	>2 min	76.5	12.4	44.3	0	0	0	0	1	0	1	1	0	0	0
1	4	5	4	3	1	> 20 m	> 2 min	> 3 min	12.6	25.4	565	0	1	1	1	1	1	1	0	0	0
0	0	0	0	0	1	< 20 m	12.6	34.1	11.2	0	130	0	1	1	1	1	1	0	0	1	0
0	3	0	0	0	1	> 15 m	17.4	34.3	12	0	657	7340	1	1	3	1	1	1	0	1	12
0	1	2	2	0	0	< 20 m	10.9	34.8	0	0	246	0	0	0	0	0	0	0	0	0	0
0	3	3	1	0	1	> 20 m	> 2 min	> 3 min	13.8	50.2	287	1385	1	1	2	1	1	1	0	0	0
0	1	1	1	0	0	< 20 m	11.8	23	1.1	0	449	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	> 20 m	> 2min	125	12.1	31	342	0	1	1	3	1	1	0	0	0	0
0	3	3	1	0	1	> 20 m	24.5	34.4	12.4	0	1550	0	1	1	1	0	1	0	0	0	0
0	0	0	0	0	0	< 15 m	9.9	39.7	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	< 20 m	11.1	30	0	0	2447	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	1	< 20 m	11	38.5	0	0	0	0	0	0	1	0	0	0	0	0	0
0	4	5	0	0	1	> 20 m	19.5	58.5	11.9	48.9	26491	0	1	0	4	1	1	0	0	1	0
0	2	2	2	0	1	> 20 m	28.3	68	12.3	51.8	142	0	0	0	3	1	1	0	1	0	0
0	0	0	0	0	1	> 20 m	31.9	34	11.3	0	0	0	0	0	1	0	1	0	0	0	0
0	3	0	0	0	1	> 20 m	>2 min	69	14	29.1	342	0	1	1	3	1	1	1	0	1	4
0	3	4	0	0	1	> 20 m	22.8	35	11.6	0	563	0	1	0	2	1	0	0	0	0	0
0	5	6	2	0	1	< 20 m	14	32	11.4	0	1277	845	0	0	4	1	0	0	1	1	6
0	4	5	2	0	1	> 20 m	Lysed	Lysed	0	0	1448	0	1	1	2	0	1	1	0	0	0

1	1	1	1	0	14	1	9	Cardiogenic shock with myocard				51	BF ASV PREMKUMAR			0.64	0.539	0.469	0.442	0.502
0	1	1	1	0	2	1	9					52	RAJA			0.528	0.51	0.451		
1	1	1	1	0	0	1	11	Plasmaj	ATN in renal biopsy with			53	RAMADAS SURESH			0.928	0.907	0.612	0.492	0.498
0	0	0	0	0	2	1	5					54	ANITA			0.467	0.406	0.388	0.546	0.548
0	1	1	1	1	5	1	7	GNB Pneumonia				55	RAMKI			0.47	0.449	0.542	0.518	0.47
0	1	1	1	0	0	1	3	Rhabdomyolysis				56	GOVINDRAJ			0.449	0.542	0.534	0.52	0.514
0	0	1	1	0	2	1	7	ASV ANAPHYLACTIC SHOCK				57	RAMESH			0.424	0.335	0.388	0.398	0.416
0	0	1	1	0	0	1	7					58	NARAYAMOORTHY			0.421				
0	0	1	1	0	0	1	6					59	ANNAPOORNA			0.294	0.311	0.361	0.357	0.294
0	1	1	1	1	0	1	9					60	PALANI			0.575	0.366	0.357	0.293	0.308
0	1	1	1	0	3	1	9					61	ARUMUGAM			0.339	0.221	0.266	0.253	0.352
0	0	1	1	1	3	1	7	Crush injury feet				62	MURUGAN			0.315	0.294	0.318	0.293	0.294
0	0	1	1	0	2	1	11					63	SATHAKUMAR			0.317	0.33	0.369	0.504	0.301
1	1	1	1	0	0	1	9					64	THULASIRAM			0.44	0.532	0.395	0.313	0.291
0	0	0	0	0	0	1	5					65	RAJENDRAN			0.215				
0	0	1	1	0	2	1	3	Delayed ophitoxemia- left cereb				66	SIVA REDDY			0.378	0.467	0.25	0.312	
0	0	0	0	0	2	1	5					67	NAVENNETHAN			0.288	0.228	0.238		
0	0	0	0	0	2	1	5					68	CHIRANJEEVI			0.33	0.335	0.296		
0	0	1	1	0	0	1	7					69	SUBHA REDDY			0.225	0.318	0.456	0.239	
0	0	1	1	0	0	1	7					70	DHANDAPANI			0.229	0.324	0.428	0.225	0.32
0	0	1	1	0	0	1	3	ASV ANAPHYLAXIS, TYPE 2 MYO				71	SUSHEELA SELVAKUMAR			0.409	0.389	0.374		
0	0	1	1	0	0	1	2					72	MEERA			0.313	0.381			
1	1	1	1	0	5	1	9	ATN in renal biopsy				73	BANU			0.403	0.529	0.155	0.214	0.182
0	0	1	1	0	0	1	7					74	VENKATACHALAN			0.413	0.335	0.347	0.411	
0	0	1	1	0	0	1	6					75	NATARAJ			0.336				
0	1	0	0	0	3	1	8	NFGNB Pneumonia				76	MAHABOOB BASHA			0.446	0.358	0.309		
0	1	1	1	1	6	1	6	Transient myocarditis, post cardi				77	VIJAYA			0.306	0.32	0.318	0.305	0.303
0	0	0	0	0	0	1	2					78	SARASWATHI			0.355				
0	0	1	1	0	0	1	3					79	SATHYA			0.358				
0	0	0	0	0	4	1	5					80	RANJIT KUMAR			0.347	0.386			
0	0	1	1	0	0	1	3					81	VALLIAMMAL			0.641	0.465	0.404		
0	0	1	1	0	0	1	6					82	MEENA			0.421	0.575	0.427		
1	1	1	1	0	9	1	9	Resolving ATN.Pseudomonas sep				83	PERUMAL			0.375	0.342	0.404	0.361	0.313
0	0	1	1	0	0	1	11					84	SARASWATHI			0.361	0.373			
0	1	1	1	0	0	1	9					85	BALARAMAN			0.347	0.341			
1	1	1	1	0	0	1	9					86	JAYANTHI			0.375	0.354			
0	1	1	1	0	0	1	9					87	BHARATHI			0.453	0.335			
0	0	1	1	0	0	1	3					88	DHANASEKAR			0.369				
0	0	1	1	0	8	1	7	Right hemiplegia, watershed infa				89	DEVANDRAN			0.709	0.545			
1	0	1	1	1	0	1	11					90	RANGAREDDY			0.306	0.287	0.305		
1	1	1	1	0	6	1	9	myocar	GNB sepsis /pneumonia			91	PANCHALI			0.288	0.306	0.311		
0	0	1	1	1	3	1	6					92	RAJA			0.401				
0	0	1	1	0	0	1	7					93	KUMAR			0.35	0.332			
0	0	1	1	0	0	1	6					94	MUNUSAMY			0.338				
0	0	1	1	0	0	1	3					95	SAKRAVARTHI			0.351	0.315	0.351		
0	1	1	1	0	0	1	7					96	MAMTHA			0.339	0.312	0.31		
0	0	1	1	0	0	1	2					97	UMA MAGESWARI			0.303	0.343	0.338		
0	1	1	1	0	0	1	2					98	RAJENDRAN			0.298				
0	0	1	1	0	0	1	3					99	CHINNAPA			0.317				
0	1	1	1	0	0	1	9					100	PARASIVAM			0.315	0.31	0.649		
0	0	1	1	0	2	1	7					101	AMUL			0.345	0.293			
0	0	1	1	0	0	1	3					102	GOPI			0.958				
1	1	1	1	0	6	1	9					103	SELVAN			0.778	0.313	0.339	0.451	
0	1	1	0	0	0	1	7					104	MOORTHY			0.277	0.314			
1	1	1	1	0	6	1	9					105	GOVARTHANA			0.246				
0	1	1	1	0	4	1	7													